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(54) Title: UNIVERSAL PRIMERS FOR WILDLIFE IDENTIFICATION

(57) Abstract: The invention provides novel universal primers that can amplify the fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and reveal the identity of the biological material of any unknown animal origin and a method for identification of the specific animal from a given biological sample.

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UNIVERSAL PRIMERS FOR WILDLIFE IDENTIFICATION TECHNICAL FIELD

The invention relates to the identification of novel universal primers that can amplify the fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and reveal the identity of the biological material of any unknown animal origin at species and sub-species sources. The invention also provides a method for the identification of fragments on mitochondrial cytochrome b gene in biological material of unknown origin.

BACKGROUND ART

A large number of studies in evolutionary biology utilize phylogenetic information obtained from mitochondrial cytochrome b gene. It has been identified a potent molecule to distinguish the phylogenetic depth of different lineages to family, genus and species in molecular taxonomy1-66. A vast database of the sequences of cytochrome b gene of different animal species has accumulated in public databases such as GenBank, NCBI (http://www.ncbi.nlm.nih.gov) etc. We have utilized this capacity of cytochrome b gene in establishing the identity of the origin of animal parts and product to its family, genus and species sources. The technique developed is based on a pair of universal primer that can amplify a small fragment of cytochrome b gene from a vast range of animal species. Establishing identity of confiscated animal parts and products is a great challenge to law enforcement agencies because none of the methods available till date is too efficient to reveal the identity of animal remains beyond a reasonable doubt. Morphological markers, described for certain species allow the identification of complete specimen of animals⁶⁷. However, a complete specimen is confiscated very rarely by the investigation agencies; therefore, these marker are not practical in wildlife forensics. The biochemical traits such as the bile characteristics⁶⁸ blood heam analysis^{69,70} etc. have also been employed in wildlife forensic for identification of individual species. The difficulty of these markers are that these markers are limited in number and are rarely found in their natural forms in which these were originally described as the characteristic of a particular species. The molecular approaches such as micro-satellite based identification⁷¹, Restriction

The molecular approaches such as micro-satellite based identification, Restriction fragment length polymorphism analysis of mitochondrial genes or PCR based species specific STS markers require the prior information of the species to establish the identity, These methods also need a significant amount of DNA material to be analysed. We may not have the prior information about the species origin of confiscated animal parts and product in forensics, therefore, these methods are not really useful and

practical in wildlife identification. The technique invented by us is universal, therefore does not require any background information to establish the identity of any unknown confiscated remains at family, genus and species sources. Being a PCR based procedure it can be applied with trace amount of any biological material. Because the amplicon length is small (472 bp); therefore, it can work perfectly with the mutilated remains, which are commonly seized by the crime investigation agencies. It does not require the large amount of genetic material i.e. DNA to be analyzed to establish the identity, hence, can detect a minute amount of adulteration in food products. The procedure described is simple and very fast. Due to the said advantages, the procedure invented by us is most suited for forensic wildlife identification.

OBJECTS OF THE INVENTION

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The main object of the invention is to identify a fragment on mitochondrial cytochrome b gene capable of significantly discriminating among various evolutionary lineages of different animal species.

Another object is to identify a fragment on mitochondrial cytochrome b gene which is flanked by the highly conserved sequences at a vast range of animal species.

Yet another object is to detect a fragment on mitochondrial cytochrome b gene which is polymorphic inter-specifically, but monomorphic at intra species sources.

20 Still another object is to develop the universal primers to amplify the fragment on mitochondrial cytochrome b gene using polymerase chain reaction.

Another object is to develop a PCR protocol that works universally with DNA template of any unknown origin (i.e. all the animal species).

Yet another object is to provide a universal method for identification of species of analyzed material (i.e. the DNA isolated from confiscated animal remain of unknown origin) using the public databases such as GenBank, NCBI etc.

Still another object is to provide a universal method of animal identification to establish the crime with the criminal beyond a reasonably doubt.

Another object is provide a universal method to establish the identity of biological materials such as skin, horns etc confiscated from animal poachers, if it is that of an endangered species.

Yet another object is to provide a universal method for establishment of the identity of confiscated animal parts and products of endangered animal species for the purpose of production of molecular evidence of animal hunting and related crime in the court of law,

35 so that the human violation to the wildlife resources could be controlled.

Still another object is to provide a universal technique to have an idea of the geographical location of the commitment of wildlife crime based on the haplotype of poached animal identified by the universal primer invented.

Another object is to provide a universal technique of animal identification to detect the adulteration of animal meat/products in vegetarian food product for the purpose of food fortification, by the food fortification agencies.

Yet another object is to provide a universal technique for detection of the origin of blood or blood stains etc collected from the scene of crime related to offences such as murder, rape etc, in order to establish the origin of blood found at scene of crime when it sounds as if criminals have wontedly spread the blood of an animal at the scene of crime, to confuse the crime investigation agencies and forensic scientists with human blood.

Another object is to invent and authenticate a universal technique that can be converted to a (a) 'MOLECUALR KIT' and (b) 'DNA CHIPS' based application to meet the requirements of above objectives.

15 SUMMARY OF THE INVENTION

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Accordingly, the invention provides novel universal primers that can amplify the fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and reveal the identity of the biological material of any unknown animal origin

DETAILED DESCRIPTION OF THE INVENTION

Keeping in view the above objectives, the cytochrome b gene sequences (1140 bp) of 221 distantly related animal species (listed in Table 1) representing various families were obtained from public database NCBI (http://www.ncbi.nlm.nih.gov). These sequences were aligned using the software Clustal X(1.8)(NCBI, USA) and a fragment (of 472 bp, alignment shown in Table 2) of gene was identified which had all the features mentioned above under column 1, 2 and 3 of sub-heading 'Objectives of invention'. As for the identity of this fragment we would like to mention that it includes the nucleotides between 398 to 869 in Antilope cervicapra and Felis catus; however, 399 to 870 in Homo sapiens sapiens species. Except at few positions (marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, giving rise to their unique molecular signature. These molecular signatures are characteristic of its species and form the basis of revealing the identity of the biological material of an unknown animal origin by the procedure invented by us. Considering Antilope cervicapra as a representative species, the sequence of this fragment is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of Antilope cervicapra:

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A pair of universal primer was designed to amplify this fragment in polymerase chain reaction (PCR). These primers were named as 'mcb398' and 'mcb869' because of its property to amplify a region of mitochondrial cytochrome b gene between nucleotides 398 to 869 of Antilope cervicapra, a representative animal species for this invention. We took this animal species as representative species because the idea of developing such a novel primers came in the mind of inventors while they were working on the genome of this animal in Centre for Cellular and Molecular Biology, Hyderabad, India. These primers work universally because its 3' end are highly conserved amongst a vast range of animal species (shown in Table 2). As mentioned above, the DNA fragment (sequence of which is shown above) targeted by these primers is highly polymorphic inter-specifically; however, it is monomorphic among the individual of same species (Tables 6, 7a, 7b, 7c, :d and 8, respectively). These unique features of the targeted region enable these primers to generate the molecular signatures of an individual species; thereby, enabling them to differentiate amongst the animals of different species (see in Figure 1c). The variation within the fragment amplified by these primers increase with increasing distances of evolutionary lineages of two animals (Table 8). These unique features of the fragment amplified by the universal primers 'mcb398' and 'mcb869' invented by the applicants fulfill the objectives of invention.

Thus, the primers invented by us can generate the molecular signature from any biological material of unknown animal origin, which actually is the characteristic of its family, genus and more precisely, the species. When these signatures are compared *in-silico* with the signatures already available in public databases (viz., GenBank, NCBI database etc) using 'BLAST software⁷³, it indicates identity of the family, genus or species of the analyzed material, which in turn is confirmed practically by comparing with the reference unimals of the revealed family, genus or species, by including them in the further analysis by the primers 'mcb398' and 'mcb869'. The complete procedure involved in the analyses (the word, 'analyses' should be understood with the stepwise procedure to establish the identity of the biological remain of any unknown animal origin for the aims mentioned in columns

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1-13 under sub-heading 'Objectives of invention') is briefed under 'Examples 5 and 6, respectively, as well as illustrated in Figures 1a, 1b and 1c, respectively.

BRIEF DESCRIPTION OF DRAWING AND TABLES

Figure 1a. Illustration of the step-wise procedure involved in analyses. The unknown biological material i.e. 'adil.flesh' refers to the confiscated skin mentioned in 'Example 6'. The arrow marks indicate the stepwise procedure involved. The brief description of Figure 1a is as follows:

The biological material i.e. the confiscated skin 'adil.flesh' was subjected to DNA isolation using the standard procedures⁷⁴. The DNA obtained was amplified using the primers 'mcb398' and 'mcb869' in PCR, fractionated in 2% (w/v) agarose gel, visualized and photographed under UV light using Gel Documentation System (Syngene, USA). The lane 'M' shown in the photograph represents the molecular weight marker (Marker XIII, Boehringer mannheim). Lane 1 shows the PCR amplicon (472 bp) obtained from 'adil.flesh' using primers 'mcb398' and 'mcb869'. The PCR amplicon obtained were sequenced at both the strand using "ABI Prism 3700 DNA Analyzes, PE-Applied Biosystems). The chromatogram shows the sequences (about 80 bp long, i.e. between 150-230 bp of sequence (328 bp), revealed from the PCR product of 472 bp length) obtained from 'adil.flesh'.

Figure 1b. Illustrates the further steps involved in analyses. The sequence (328 bp) revealed from 'adil.flesh' was subjected to homology search in nr (i.e. non-redundant) database of Netional Centre for Biological Information (NCBI), USA. The sequences producing significant alignments are shown along with its bits score and E values. It indicates the extent of homology amongst the sequence enquired (i.e. the 328 bp sequence from adil.flesh) and the sequences registered in nr database of NCBI. BLAST analysis revealed the highest homology of the sequence revealed from 'adil.flesh' with the sequence of Panthera pardus (gene bank registration number 'AY005809'), indicating the identity of adil.flesh as that of a leopard (Panthera pardus) origin. Figure 1b further illustrates the multiple alignments of the sequences obtained from reference animals (listed in Table 5) along with the sequence obtained from 'adil.flesh'. The sequences of 'adil.flesh' is similar to the sequences of 'gz1L' further confirming the identity of the source of confiscated remain 'adil.flesh' as that of a Panthera pardus origin.

Figure 1c illustrates the NJ-tree (Neighbor Joining tree) constructed using CLUSTAL X (1.8) from the sequences revealed from 'adil.flesh' and reference animals listed in Table 5.

The animals belonging to similar species cluster together; however, the animals of different species group in different clusters. The confiscated material under investigation (i.e. 'adil.flesh') clusters with 'gz1L' (i.e. the known normal leopard 'Panthera pardus') indicating the identity of the species of 'adil.flesh' as that of a Panthera pardus source.

- Figure 2 shows the Agarose gel electrophorogram showing the PCR amplicons (472 bp) obtained from the reference animals of family felidae listed in Table 5, using universal primers 'mcb398 and 'mcb869'. Description of different lanes is as follows:
 - Lanes 1-21: The PCR profiles of the animals 1-21, respectively, listed in Table 5.
- Lane 22: The PCR profiles of DNA isolated from confiscated skin of unknown animal origin 'i.e. adil.flesh'
 - Lane 23: Negative control (no DNA)
 - Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)
 - Figure 3. Shows PCR amplicons obtained from animals listed in Table 9. The primers used in PCR are 'AFF' and 'AFR'. The description of different lanes shown is as follows:
- Lane 1-4: The PCR profiles of animals 1-4, respectively, listed in Table 9, showing amplicons of 354 bp.
 - Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)
 - Figure 4. Shows PCR amplicons obtained from animals listed in Table 12. This experiment demonstrates the universal nature of our primers among a vast range of animal
- 20 species. Description of different lanes shown is as follows:
 - Lanes 1-23: The PCR profiles of the animals 1-23, respectively, listed in Table 12. The PCR product of 472 bp is amplified universally from all the animal species analyzed.
 - Lane 24: Negative control (no DNA)
 - Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)
- Table 1. List of 221 animal species used for *In-silico* analysis to design the universal primers 'mcb398' and 'mcb869'. Table also demonstrate the 'P,S scores' of 'mch398' and 'mcb869' for different templates. The descriptions of various symbols used in this table are as follows:
 - Symbol (#) refers to Number
- 30 Symbol (*) refers to the animal species which is either protected species (listed in Wildlife (Protection) Act, 1972 (Central Act NO 53 of 1972), or an endangered/rare animal species Symbol (⁵P,S/F) refers to Probability of match and Stability of match of primer 'mcb398' with different templates (i.e. the cytochrome b gene from different species origin). A higher P, S score refers to the higher probabilities of significant amplification of specific

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template by the primer. It is calculated by Amplify (1.2) software.

Symbol ($^{\Psi}P,S/R$) refers to <u>Probability</u> of match and <u>Stability</u> of match of primer 'mcb869' with different templates. A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify* (1.2) software.

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Table 2. Multiple sequence alignment of 472 bp fragment of mitochondrial cytochrome b gene (identified by inventors to fulfill the requirements of column 1, 2 and 3 mention under sub-heading 'Objectives of invention') of 221 animal species listed in Table 1. Alignments also show the binding sites for universal primers 'mcb398' and 'mcb869'. The symbol (*) refers to the nucleotide bases which are conserved amongst 221 animal species listed in Table 1). The alignments have been done using software CLUSTAL X (1.8). The nucleitide positions that are unmarked are variable amongst 221 animal species analyzed. These variable sites together constitute the molecular signature of an individual species, giving rise to molecular basis of species identification by our primers.

Table 3. Results of the blast analysis of the sequence revealed from 'adil.flesh' in 'mito' database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece 'adil.flesh' with felis catus cytochrome b gene sequence (genbank registration number NC_001700.1, bits score 365, E value, e-101) registered in NCBI database (bits score 365 and E value e-101). It gives an indication that the species of analyzed material belongs to family felidae. It also fulfills the requirements of column 6 mention above under sub-heading 'Objectives of invention'.

Table 4. Results of the blast analysis of the sequence revealed from 'adil.flesh' in 'nr' database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece 'adil.flesh' with Panthera pardus cytochrome b gene sequence (genbank registration number AY005809, bits score 603, E value, e-170) registered in NCBI database. It gives an indication that the species of analyzed material belongs to Panthera paurdus origin. It also fulfills the requirements of column 6 mention above under sub-heading 'Objectives of invention'.

Table 5. Reference animal belonging to family felidae selected for comparison with 'adil.flesh' to confirm the findings of BLAST analysis results of which are mentioned in Table 3 and 4, respectively. The animals listed in SN. 1-21 represent different species of family felidae. SN. 22 and 23 are primate species taken for out-group comparisons.

Table 6 Multiple sequence alignments of cytochrome b sequences (328 bp) revealed from 'adil.flesh' and reference animals listed in Table 5. The positions that have a common nucleotide in all the animal species under investigation are shown with a star (*) mark;

however, the positions that are variable in any of the animals under investigation are unmarked. The nucleotides at these positions constitute the molecular signature of an individual species, which are unique and highly specific for its species. These signatures are the molecular basis of identification of individual animal species using our primers 'mcb398' and 'mcb869'.

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Table 7 (Tables 7a, 7b, 7c and 7d). The comparison of the molecular signatures of different animal species investigated along with 'adil.flesh', the confiscated skin of unknown animal origin. This table demonstrates the variable positions (i.e. the positions which are not marked with star (*) symbol in Table 6), amongst the 328 bp fragment revealed from the animals listed in Table 5. The dot (.) mark represents the presence of the similar nucleotide as listed in lane 1 i.e. the sequence from "adil flesh' at that position. It demonstrates that the signatures of each species are unique and specific to its species. The molecular signatures of 'adil.flesh' are comparable (except for position 37 which has a transition from 'T' to 'C') to the molecular signature of 'gz1L' i.e. the known leopard 'Panthera pardus' source, indicating the identity of the source of confiscated skin 'adil.flesh' as that of a leopard 'Panthera pardus' source. The nucleotide variations (at the positions 153, 198, 223, 264, among the known leopards, (i.e. gz1L, gz2L, and gz3L, respectively)), give an idea about the geographical habitat of each animals. Various studies referring to molecular evolution of different animal species support this hypothesis⁷⁵; however, it could further be confirmed by taking the reference animals from different geographical areas and analyzing by our primers 'mcb 398' and 'mcb869'. If we could generate the database of different haplotypes (i.e. habitat specific molecular signatures) of the animal species, it would also enable our primers to reveal the geographical location of the commitment of wildlife crime.

Table 8. Percent similarity matrix calculated by pair-vise comparisons of nucleotide sequences aligned (illustrated in Table 6). The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity (99.7% and 98.2%, with the lineages of animals 'gz2L' and 'gz3L', respectively) with the sequences obtained from known normal leopard source, indicating its identity as that of a leopard origin. The similarity matrix has been calculated using the software PHYLIP (3.5).

Table 9. Animals selected for validation of minimum P,S score for efficient amplification of cytochrome b gene of different origin by the primers 'mcb398' and 'mcb869'. P,S score of primers 'AFF' and 'AFR' for these animals are shown.

Table 10. BLAST analysis of primers 'mcb398' in nr database of NCBI. It demonstrates

that the 3' end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer Table 11. BLAST analysis of primers 'mcb869' in nr database of NCBI. It demonstrates that the 3' end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer.

Table 12. Other animal belonging to distantly related animal species, investigated to confirm the universal nature of primers 'mcb398' and 'mcb869'. Gel photograph showing the PCR amplicons from these animals are shown in Figure 4.

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The mitochondrial cytochrome b gene has very widely been used in molecular taxonomic studies. It has immense capabilities to reveal different evolutionary lineages of animals in family, genus and species specific manner. It has also been used to classify the population of a particular species according to its demographic distributions⁷⁵. The vast database of cytochrome b sequences of different animal species has accumulated in public databases such as Genbank and NCBI¹⁻⁶⁵. We have explored these unique characteristics of cytochrome b gene to establish the identity of confiscated remains of any unknown animal by inventing a pair of novel primers, 'mcb398' and 'mcb869', that can amplify a small fragment (472 bp) of cytochrome b gene of wide range of animal species in universal manner. These primers work universally because its 3' ends target within a highly conserved region.

The fragment of cytochrome b gene identified had all the features mentioned in columns 1, 2 and 3 listed under sub-heading 'Objective of invention'. We identified this fragment by aligning the cytochrome b gene sequences (1140 bp) of 221 different animal species listed in Table 1. These sequences are publicly available in NCBI DNA databases. These sequences were aligned using the software CLUSTAL X (1.8). As mentioned before, the 472 bp fragment of cytochrome b gene identified by us to have the features mentioned in columns 1, 2 and 3 listed under sub-heading 'Objective of invention' includes the nucleotides between 398 to 869 in Antilope cervicapra and Felis catus; however, 399 to 870 in Homo sapiens sapiens species. Except at few positions (marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, revealing the identity of the biological material belonging to that of an unknown animal origin by the procedure invented by us. As for identity of this fragment we are

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considering Antilope cervicapra as a representative species, and the sequence the above fragment of cytochrome b gene of Antilope cervicapra is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of Antilope cervicapra

"taccatgaggacaaatatctttttgaggagcaacagtcatcaccaatctcctttcagcaatcccatacatcggtacaaacctagtaga atgaatctgaggagggttctcagtagataaagcaacccttacccgatttttcgccttccactttatcctcccatttatcattgcagccctt accatagtacacctactgtttctccacgaaacaggatccaacaaccccacaggaatctcatcagacgcagacaaaa:::cnticcac ccctactacactatcaaagatatcctaggaggctctactattaattttaaccctcatgcttctagtcctattctcaccggacctgcttggag acccagacaactatacaccagcaaacccacttaatacacccccacatatcaagcccgaatgatacttcctatttgcatacgcaatcct ccgatcaattcctaacaaactaggagg

Table 2 presents the alignment of the above fragment of cytochrome b gene of 221 animal species. Each species in table 2 has been represented by a unique code, which is decoded in Table 1. We selected these species to represent the vast range of animal families of distant orders. Of 221 species, about 65 were the protected/endangered or rare species listed in Wildlife (Protection) Act, 1972 (Central Act NO 53 of 1972). These species are marked with symbol (*) in Table 1. The NCBI accession number refers to its registration number in NCBI database and the number in superscript represent the reference cited. Based on the aligned cytochrome b sequences of different 221 animal species the primers designed were as follow:

Primers name

Sequence (5'-3')

20 'mcb398'

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"TACCATGAGGACAAATATCATTCTG"

'mcb869'

"CCTCCTAGTTTGTTAGGGATTGATCG"

Tables 2, 10 and 11, respectively, demonstrates that the 3' ends of the primers are highly conserved amongst all the animal species analyzed *in-silico* (In total 221 animal species listed in Table 1 and about 500 species listed in Tables 10 and 11, respectively) Also, the 5' end of the primers were selected within the conserved region of cytochrome b gene to improve the probability and stability of match of the primers to their target sequences (i.e. the above mentioned 472 bp fragment of cytochrome b gene). The primers were thoroughly checked for internal stabilities, loop or dimmer formation using different software viz., 'Amplify (1.2)', 'Primer3' (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) as well as manually. We assigned the P,S score (P=Probability of match, S=Stability of match) to the primers for each template using the software Amplify (1.2). The higher scores of P and S ensure a good amplification if all other conditions standard (which are mentioned under 'Example 3') are optimum. The Highest score for 'mcb398' was 98.63 (i.e. the situation where the primer has perfect match with template);

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however, the highest P, S for 'mcb869' was recorded as 98, 68 for a complete match between the primer and template. The lowest P,S score observed for 'mcb398' was 81,50 for species Talpa europaea whereas 'mcb869' had a high P, S score for this species (92, 57). The another species which have lowest P, S score for one of the two primers were Eumeces egregious and Equus ainus. Eumeces egregious had P, S score 86, 55 and 73,51 for 'mcb398' and 'mcb869', respectively; however, the P, S score of Equus ainus was calculated as 91,61 and 73, 51 for 'mcb398' and 'mcb869', respectively. All other animals had higher P, S scores then the above mentioned species. To ensure that these primers would work efficiently with the DNA template from the animals having the lowest P, S score for one of the primers, we designed an another experiment to validate the lower limits of one of the two primers sufficient for efficient amplification in PCR. We designed (AFF= 5'tagtagaatgaatctgaggagg3' and another primer pair an AFR=5'atgcaaataggaagtatcattc3'.) having more mis-pairing at their annealing sites (but not at ends), therefore have less internal stability and lower P, S scores for its templates (listed in Table 9). The P,S scores of 'AFF' and 'AFR' were as calculated as low as 41 and 49 for Platanista gangetica and Sus scrofa These species were amplified efficiently using the primers 'AFF' and 'AFR' (results shown in Figure 3) (keeping all other conditions standard i.e. the conditions mentioned in 'Example 3'). The lowest P,S scores (86, 55 and 73,51 for species Eumeces egregious) for our primers 'mcb398' and 'mcb869', respectively, were higher then the above range of combined P, S scores of 'AFF' and 'AFR' for species Sus scrofa (87, 52 and 87, 41), which was efficiently amplified by the primers 'AFF' and 'AFR'. It gives an indication that the primers 'mcb 398' and 'mcb 869' would work with all the species including Eumeces egregious efficiently to give rise to the expected product in PCR. This experiment confirmed that the primers 'mcb398' and 'mcb 869' are capable of amplifying the cytochrome b fragment of most of the animal species in a universal manner.

For further confirmation of universal nature of our primers, we blasted the sequence of our primers against the *mito* and *nr* databases of NCBI using BLAST software. The results of these analyses are shown in Tables 10, and 11, respectively.

Finally, the universal nature of the primers was tested in our laboratory with some more animal species listed in Table 12. These primers amplified all the animal species efficiently, giving rise to the band of expected size (472 bp). The results are shown in Figure 4. This experiments substantiated the results of P.S analysis and other *in-silico* analyses to show that the primers 'mcb398' and 'mcb 869' are universal primers.

The flow chart of establishing identity of the species of biological material of unknown animal origin using primers 'mcb398' and 'mcb869'

Biological material of unknown animal origin 5 DNA isolation PCR amplification of DNA isolated using primers 'mcb398' and 'mcb869' 10 Sequencing at both the strands in triplicate (using any standard procedure of sequencing such as using ABI Prism 3700, PE-Applied Bio-systems) BLAST of revealed sequence in mite database of NCBI (http://www.ncbi.nlm.nih.gov/BLAST 15 (it gives idea about the family of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database) BLAST of revealed sequence in nr database of NCBI (http://www.ncbi.nlm.nih.gov/BLAST 20 (it gives idea about the Genus or more precisely, species of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database) Selection of reference animals belonging to the family/Genus/and species revealed by mito and nr BLAST 25 Isolation of DNA from the blood of known reference animals; PCR amplification using primers 'mcb398' and 'mcb869'; sequencing of the PCR products in triplicate using the same primers 30 Multiple sequence alignments of the revealed sequences of mitochondrial cytochrome b gene of known reference animals and the biological material of unknown animal origin using software such as Autoassembler (/CLUSTAL X (1.8) 35 Identification of sequence from the aligned sequences

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that is homologous (or significantly similar) to the cytochrome b gene sequence of the DNA obtained from biological material of unknown animal origin.

The species of homologous sequence would be the species of the biological material under investigation

Examples

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Example 1

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Example for identification of a fragment of cytochrome b gene fulfilling the requirements of columns 1, 2 and 3 mentioned under sub-heading 'Objectives of invention' of heading 'Brief summary of invention'

The cytochrome b molecule has very vastly been used in molecular taxonomic studies. Being a slow evolving gene, It has a tremendous information in its nucleotide sequences to distinguish the animals to their family, genus and species sources 1-65. A vast database of the sequences of cytochrome b gene of different animal species has accumulated in the nr and mito databases of NCBI. We have explored these qualities of cytochrome b gene to establish the identity of confiscated remains of unknown animal origin to its family, genus and species sources. For this purpose, we have identified a fragment of cytochrome b gene which is highly polymorphic inter-specifically, however, it is monomorphic among the individual of same species, therefore it can group the individual of an unknown species with the known individuals of reference species to which it belongs. In order to amplify this fragment from DNA isolated form any unknown origin, it was necessary that it remain flanked with the highly conserved sequences amongst a vast range of animal families. To identify such a unique fragment within the cytochrome b gene, we aligned the sequences of 221 distantly related animal species (listed in Table 1) representing various families using software CLUSTAL X (1.8). These sequences were obtained from public database NCBI (http://www.ncbi.nlm.nih.gov). The aligned data was examined carefully for the conserved sites amongst all the species included in in-silico analysis. We identified a fragment (472 bp) of cytochrome b gene that was fulfilling all the requirements mentioned above and also under column 1, 2 and 3 of sub-heading 'Objectives of invention'.

As for the identity of this fragment we would like to mention that it includes the nucleotides between 398 to 869 in Antilope cervicapra and Felis catus; however, 399 to 870 in Homo sapiens sapiens species. Except at few positions marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, giving rise to their unique molecular signature. These molecular signatures are characteristic of its species and form the basis of revealing the identity of the biological material of an unknown animal origin by the procedure invented by us. Considering Antilope cervicapra as a representative species, the sequence of this fragment is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of Antilope cervicapra

"taccatgaggacaaatatctttttgaggagcaacagtcatcaccaatctcctttcagcaatcccatacatcggtacaaacctagtaga atgaatctgaggagggttctcagtagataaagcaacccttacccgatttttcgccttccactttatcctcccatttatcattgcagccctt accatagtacacctactgtttctccacgaaacaggatccaacaaccccacaggaatctcatcagacgcagacaaaattccattccac ccctactacactatcaaagatatcctaggagctctactattaattttaaccctcatgcttctagtcctattctcaccggacctgcttggag acccagacaactatacaccagcaaacccacttaatacacccccacatatcaagcccgaatgatacttcctatttgcatacgcaatcct ccgatcaattcctaacacaaactaggagg"

Example 2:

Example for development of universal primers to amplify the fragment identified mentioned under 'Example 1'.

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A pair of universal primer was designed which has the following features:

- 1. It targets the fragment identified (mentioned under 'Example 1') to amplify it in polymerase chain reaction (PCR).
- 2. Its 3' and 5' ends that are highly conserved (marked as star (*) in Table 2), amongst a vast range of animal species ensuring the amplification of the fragment mentioned above in a universal manner. The sequencing of the fragment amplified by these primes reveals the molecular signature of the species of analyzed material, which on comparison with the sequences of the known reference animals reveals the identity of the species of unknown biological material under investigation.
- 3. The tm (melting temperature) of both primers was almost similar (about 58 degree centigrade) ensuring the significant annealing of both the primers to its template, therefore significant amplification of targeted region in PCR.
 - 4. The internal stability and P, S, score of the primers were ensured higher while designing it. The possibilities of internal loop formation, dimmer formation etc were also excluded by selecting its sequence uniquely. This ensured that the primer would be a good primer to be used in PCR for amplification of DNA from unknown animal origin.
 - 5. The 3' end of the primers were ensured to have either 'G' or 'C' to increase the probability of strong bonding at its 3'ends, which is necessary for efficient amplification of DNA template in PCR. It also strengthens the universal nature of the primer.
 - 6. The sequences of the primers were ensured to be unique so that it does not give rise to non-specific and spurious products in PCR leading to confusion. It improved the efficiency and quality of the technique invented by us.

7. These primers were named as 'mcb398' and 'mcb869' because of its property to amplify a region of mitochondrial cytochrome b gene between nucleotides 398 to 869 of Antilope cervicapra, a representative animal species for this invention. We took this animal species as representative species because the idea of developing such a novel primers came in the mind of inventors while they were working on the genome of this animal in Centre for Cellular and Molecular Biology, Hyderabad, India.

8. The sequences of the universal primers invented are as follows:

Primers name

Sequence (5'-3')

10 'mcb398'

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"TACCATGAGGACAAATATCATTCTG"

'mcb869'

"CCTCCTAGTTTGTTAGGGATTGATCG"

Example 3:

Example for development of universal PCR conditions to ensure the amplification of a template of any unknown origin in PCR, hence strengthening the universal nature of the technique invented by us

The PCR conditions developed had the following unique features:

- 1 These were capable of amplifying the DNA template of any animal origin in an universal manner using the universal primers mentioned under 'Example 2'.
- 2. The conditions were selected to ensure the comparable annealing temperature for both the primers i.e. 'mcb398' and 'mcb869'.
 - 3. The PCR conditions standardized herewith are universal; therefore, the possibility of PCR failure with a template of unknown origin due to non-standard conditions is excluded. It ensures the universal nature of our technique to be used in wildlife forensics.
 - 4. The universal conditions mentioned above are:

Amplification reactions should be carried out in 20 µl reaction volume containing approximately 20 ηg of template DNA, 100µm each of dNTPs, 1.25 pmole of each primer, 1.5mM MgCl₂, 0.5 unit of Ampli*Taq* Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed should be: an initial denaturation at 95°C for 10 min, followed by 35 cycles each of denaturation at 95°C for 45 s, annealing at 51°C for 1 min, and extension at 72°C for 2 min. The extension step at 35th cycles should be held for 10 min.

Example 4:

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Establishing the universal nature of our primer and experimental evidences to demonstrate the universal nature of primers:

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The universal nature of the primers 'mcb398' and 'mcb 869' was ensured by the following measures:

(a) Selecting the primers from the aligned cytochrome b gene sequences of 221 animal of distantly related species:

The cytochrome b gene sequences (1140 bp) were aligned using software CLUSTAL X (1.8). The region of cytochrome b gene that was most conserved amongst 221 animal species was selected to design the primers.

(b) Selecting the 3' and 5' ends of the primers at the highly conserved positions of cytochrome b gene:

The 3' and 5' ends of the primers were ensured to anneal to a highly conserved position amongst 221 animal species representing a vast range of animal families. It was done to ensure an efficient amplification of all the species in PCR. These positions are shown with star (*) mark in Table 2.

(c) Ensuring either 'G' or 'C' at the 3' end of the primers:

It was ensured the primers to have either 'G' or 'C' at its 3' ends as these are the nucleotides that ensure the strong bonding at the 3' ends of the primers due to three hydrogen bonds while pairing with each other. The strong bonding at 3' ends helps the primers to anneal properly with its template resulting in significant amplification in PCR.

(d) Selecting the sequences of the primers to ensure a higher internal stability, higher P, S score, and no primer dimmer and loop formation:

The sequences of the primers were selected to have a high P, S score for a vast range of animal species (Shown in Table 1). The care was taken to exclude the possibilities of loop or primer dimmer formation that could reduce the efficiency of the primers in PCR.

(e) Selecting the sequence of the primers with a comparable melting temperature:

The sequences of the primers were selected to have a comparable melting temperature so that these could work together to amplify a DNA template in PCR at a similar annealing temperature. The melting temperature of both the primers was about 58 degree centigrade and the annealing temperature used in PCR is 51 degree centigrade.

Experimental evidences to demonstrate the universal nature of primers:

(1) Evidence from In-silico analysis:

(a) Selecting the primers within the most conserved region of mitochondrial cytochrome b gene

As mentioned above, the primers were designed to anneal within a highly conserved region of mitochondrial cytochrome b gene fragment of 472 bp. Table 2 presents the alignment of the above fragment of cytochrome b gene of 221 animal species representing a vast range of animal families. The conserved positions of nucleotide sequences are shown with star (*) mark in Table 2

Table 2 also demonstrates that the 3' ends of the primers are highly conserved amongst all the animal species analyzed *in-silico*. In the aligned sequences, the conserved nucleotides are marked with symbol (*). Also, the 5' end of the primers were selected within the conserved region of cytochrome b gene to improve the probability and stability of match of the primers to their target sequences (i.e. the above mentioned 472 bp fragment of cytochrome b gene). The primers were thoroughly checked for internal stabilities, loop or dimmer formation using different software viz., 'Amplify (1.2)', 'Primer3' (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) as well as manually.

(b) P, S, score analysis:

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We assigned the P,S score (P=Probability of match, S=Stability of match) to the primers for each template using the software Amplify (1.2). The higher scores of P and S ensure a good amplification if all other conditions standard (which are mentioned under 'Example 3') are optimum. The Highest score for 'mcb398' was 98,63 (i.e. the situation where the primer has perfect match with template); however, the highest P, S for 'mcb869' was recorded as 98, 68 for a complete match between the primer and template. The lowest P,S score observed for 'mcb398' was 81,50 for species Talpa europaea whereas 'mcb869' had a high P, S score for this species (92, 57). The another species which have lowest P, S score for one of the two primers were Eumeces egregious and Equus ainus. Eumeces egregious had P, S score 86, 55 and 73,51 for 'mcb398' and 'mcb869', respectively; however, the P, S score of Equus ainus was calculated as 91,61 and 73, 51 for 'mcb398' and 'mcb869', respectively. All other animals had higher P, S scores then the above mentioned species. To ensure that these primers would work efficiently with the DNA template from the animals having the lowest P, S score for one of the primers, we designed an another experiment to validate the lower limits of one of the two primers sufficient for amplification in PCR. We designed an another primer (AFF= 5'ctagtagaatgaatctgaggagg and AFR= 5'tatgcaaataggaagtatcattc3'.) that have more mis-pairing at their annealing sites (but not at ends), therefore have less internal stability

and lower P, S scores for its templates (listed in Table 9). The P,S scores of 'AFF' and 'AFR' were as calculated as low as 41 and 49 for Platanista gangetica and Sus scrofa These species were amplified efficiently using the primers 'AFF' and 'AFR' (results shown in Figure 3) (keeping all other conditions standard i.e. the conditions mentioned in 'Example 3'). The lowest P,S scores (86, 55 and 73,51 for species Eumeces egregious) for our primers 'mcb398' and 'mcb869', respectively, were higher then the above range of combined P, S scores of 'AFF' and 'AFR' for species Sus scrofa (87, 52 and 87, 41), which was efficiently amplified by the primers 'AFF' and 'AFR'. It gives an indication that the primers 'mcb 398' and 'mcb 869' would work with all the species including Eumeces egregious efficiently to give rise to the expected product in PCR. This experiment confirmed that the primers 'mcb398' and 'mcb 869' are capable of amplifying the cytochrome b fragment of most of the animal species in a universal manner.

© BLAST analysis:

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The sequences of primers 'mcb398' and 'mcb869' were blasted against mito and nr 15 databases of NCBI to see its significant alignments with the sequences registered in GenBank. As expected, the most significant alignments of the sequences were found with the cytochrome b gene regions (within the 472 bp fragment mentioned in 'Example 1') of different animal species. This analysis also showed that the 3' as well as 5' ends of the primers were highly conserved amongst a vast range of animal species, confirming the 20 universal nature of the primers (Tables 10 and 11, respectively)

(2) Evidence from bench work/experiments done in laboratory conditions:

The DNA from different animals belonging to distantly related species (mentioned in Table 12) was isolated and subjected to PCR amplification using the primers invented by us i.e. the primers 'mcb398' and 'mcb869' The PCR products amplified were resolved in agarose gel by electrophoresis and visualized under UV light. The PCR products of expected size (472bp) were obtained from all the animals confirming the universal nature of our primers. These results are shown in Figure 4.

Example 5:

Example to establish the identity of confiscated remains from unknown animal origin using the universal primers 'mcb398' and 'mcb869'.

The step-vise procedure to establish the identity of the biological material from an unknown animal source is mentioned below:

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Biological material of unknown animal origin DNA isolation

PCR amplification of DNA isolated using primers 'mcb398' and 'mcb869'

Sequencing at both the strands in triplicate (using any standard procedure of sequencing such as using ABI Prism 3700, PE-Applied Bio-systems)

BLAST of revealed sequence in *mito* database of NCBI (http://www.ncbi.nlm.nih.gov/BLAST

(it gives idea about the family of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database)

BLAST of revealed sequence in nr database of NCBI
(http://www.ncbi.nlm.nih.gov/BLAST)

(it gives idea about the Genus or more precisely, species of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database)

Selection of reference animals belonging to the family/Genus/and species revealed by mito and nr BLAST searches

Isolation of DNA from the blood of known reference animals;

PCR amplification using primers 'mcb398' and 'mcb869'; sequencing of the PCR products in triplicate using the same primers

Multiple sequence alignments of the revealed sequences of mitochondrial cytochrome b gene of known reference animals and the biological material of unknown animal origin using software such as Autoassembler/CLUSTAL X (1.8)

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Identification of sequence from the aligned sequences

that is homologous (or significantly similar) to the cytochrome b gene sequence of the

DNA obtained from biological material of

unknown animal origin.

The species of homologous sequence would be the species of the biological material under investigation

Application of the above information for the objectives mentioned in columns 7-13 under

sub-heading 'Objective of invention' of heading 'Summary of invention'

Example 6:

The actual execution of the technique invented

As a first application and to demonstrate the ease and utility of this method, we investigated a case of forensic identification submitted at our laboratory to seek scientific opinion on animal hunting evidence. In this case, we received the half burned remains of an unknown animal, confiscated by the crime investigation agencies. The DNA was isolated from the above material following standard methods⁷⁴ and subjected to PCR amplification using the primers mentioned above (viz., 'mcb398' and 'mcb869'). Amplification reactions were carried out in 20 µl reaction volume containing 20 ηg of template DNA, 100µm each of dNTPs, 1.25 pmole of each primer, 1.5mM MgCl₂, 0.5 unit of AmpliTaq Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed were: an initial denaturation at 95°C for 10 min, followed by 35 cycles each of denaturation at 95°C for 45 s, annealing at 51°C for 1 min, and extension at 72°C for 2 min. The extension step at 35th cycles was held for 10 min.

The PCR products obtained were sequenced in automated work station (ABI Prism 3700, PE-Biosystems) on both strands in triplicate and the sequence resolved (328 bp, shown in Figure 1a) was blasted against mito databases of NCBI using BLAST program⁷³. The most significant alignment (bits Value 365, E value e⁻¹⁰¹) of this sequence was produced with the cytochrome b gene sequence of Felis catus, (Table 3) indicating that species of

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analyzed material belongs to family felidae. Further, the above sequence revealed from the confiscated remain was blasted against nr databases of NCBI using BLAST program. The most significant alignment (bits Value 603, E value e-170) of this sequence was produced with the cytochrome b gene sequence of Panthera pardus (Table 4), indicating the identity of the analyzed material as that of a Panthera pardus source. Based on this information, we selected the reference animals listed in Table 5 representing different species and subspecies of felidae. The DNA isolated from reference animals was amplified and sequenced on both strands in triplicate using the primer pair mentioned above. Consensus sequences obtained were aligned using program CLUSTAL X (1.8) (Table 6). Sequence comparisons identified 113 variable sites in total amongst all animals analyzed (Table 7). Pair-vise comparisons of sequences were performed to find out the variation among different animals investigated. All the species investigated were differentiated by a their unique nucleotides sequences. The molecular signatures of different reference animals were compared with the molecular signature of the confiscated skin 'adil.flesh'. Table 7 demonstrate that the maximum similarity of the adil.flesh with 'gzll' i.e. known Leopard (Panthera pardus) species, indicating the identity of the adil.flesh, the confiscated skin, as that of a Panthera pardus origin. We also calculated the similarity matrix showing the pairvise similarity amongst the animal species under investigation using PHYLIP software This matrix is shown in Table 8. It demonstrates that the animals belonging to different species had more variation; however, the animals of same species had maximum similarity among their cytochrome b sequences. The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity with the sequences obtained from known Leopard source(99.7%, and 98.2 with 'gz11' and 'gz21', respectively); establishing the identity of the source of confiscated material as that of a Normal leopard (Pentheru pardus) species. The step-vise procedure involved in above analysis is illustrated in Figure la, lb and lc, respectively.

Thus, the primers invented by us can generate the molecular signature from any biological material of unknown animal origin, which actually is the characteristic of its family, genus and more precisely, the species. When these signatures are compared *in-silico* with the signatures already available in public databases (viz., GenBank, NCBI database etc) using *BLAST* software⁷³, it indicates identity of the family, genus or species of the analyzed material, which in turn is confirmed practically by comparing with the reference animals of the revealed family, genus or species, by including them in the further analysis by the primers 'mcb398' and 'mcb869'. Application of the information revealed could be in

fulfilling the requirements of objectives mentioned in columns 7-13 under sub-heading 'Objective of invention' of heading 'Summary of invention'

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The method of the invention can be used to establish the identity of confiscated animal parts and products is one of the key requirements of wildlife identification in forensics. It is needed to establish the crime with the criminal beyond a reasonable doubt to avoid the human violation of wildlife resources. Various morphological biochemical and molecular approaches have been given for this purpose; however, none of the current methods is universally applicable to detect the mutilated animal remains of unknown origin. We have identified a fragment on the mitochondrial cytochrome b gene, which has enormous information to differentiate among various animal species back to the family, genus and species sources. We have also found that this fragment is flanked by the highly conserved sequences amongst a vast range of animal species. We invented a pair of universal primer that can amplify this fragment of DNA isolated from the biological material of an unknown animal origin in polymerase chain reaction (PCR) to reveal its identity at species and sub-species sources. This novel invention has great potential to revolutionize the whole scenario of wildlife forensic identification and crime investigation.

Table 1. The animal species included in the study for in-silico analysis

	SN.	Code	Name	NCBI accession #	P,S/F	⁵P,S/R
	ı	aep.mel	Aepyceros melampus	AF0362891	97, 60	94, 62
	2	•	Oreotragus oreotragus	AF036288'	88, 52	94, 62
		ore.ore	Addax nasomaculatus	AF0347222	97, 60	95, 66
	3	add.nas		AJ2226851	90, 58	95, 66
	4	ory.dam	Oryx damah Hippotragus equinus	AF0220603	98, 63	85, 55
	5	hip.equ	Alcelaphus buselaphus	AJ222681	97,60	98, 68
	6	alc.bus	Sigmoceros lichtensteinii	AF0349674	97,60	98, 68
	7	sig_lic	Beatragus hunteri	AF0349684	97,60	94, 62
	8	bea.hun	Damaliscus lunatus	.AF0166353	97,60	77, 55
	9	dam.lun	Connochaetes taurinus	AF0166383	82, 56	93, 62
		con.tau		Y150051	90, 58	87, 63
	11	bis.bon	Bison bonasus	AF0916316	90, 58	94, 62
	12	bos.gru	Bos grunniens*	AJ2226791	90, 58	95, 66
	13	bos.tra	Bos tragocamelus*	D34637 ⁷	97, 60	93, 64
		buba.bub	Bubalus bubalis •	D82895 ⁴	97, 60	87, 62
		bub.min	Bubalus mindorensis	AF091633 ⁶	97,60	87, 63
		tra.ang	Tragelaphus angasii	AF036276 ^t	90, 58	97, 64
	17	tra.eur	Tragelaphus eurycerus	U17861*	95, 6l	93, 59
		nem.cau	Nemorhaedus caudatus*	AF034732 ²	89, 55	89, 59
		pse.nay	Pseudois nayaur	AF034732 AF034731 ²	94, 58	97, 63
		amm.ler	Ammotragus lervia		98, 63	95, 66
	21	cap.fal	Capra falconeri*	D84202 ¹⁰	98, 63	89, 58
		cap.ibe	Capra ibez*	AF03473 <i>5</i> ² AF034733 ²	95, 61	90, 61
		hem.jem	Hemitragus jemlahicus*		95, 61	89, 59
	24	rup.pyr	Rupicapra pyrenaica	AF034726 ² AF034725 ²	95, 61	94, 64
		rup.rup	Rupicapra rupicapra	AF034724 ²	98, 63	95, 66
	26	pan.hod	Pantholops hodgsoni		90, 53	95, 66
	27	bud.tax.tax	Budorcas taxicolor taxicolor*	U17868° AF034727²	98, 63	97, 64
	23	ovi.amm	Ovis ammon*	AF034727 AF034729 ²	98, 63	97, 64
	29	ovi.vig	Ovis vignei*	AJ304502 ¹¹	98, 63	94, 63
	30	cap.cri	Capcornis crispus •	U17862°	98, 63	92, 61
•	31	ovi.mos	Ovibos moschatus	AF19063212	98, 63	94, 62
		ore.ame	Oreamnos americanus	AF091634	97, 58	90, 61
	33	cep.dor	Cephalophus dorsalis	AF09662913	97,60	88, 53
		cep.max	Cephalophus maxwellii	AJ00002614	95, 61	93, 59
	35 36	alc.alc hyd.ine	Alces alces	AJ000028 ¹⁴	97,60	90, 63
	37	mun.mun	Hydropotes inermis Muntiacus muntjak*	AF042718"	90. 58	93, 64
	38	cer.ele.kan	Cervus elaphus kansuensis*	AB0210981*	98, 63	82, 59
	39	cer.ele.xan	Cervus elaphus xanthopygus •	AB0210971*	98, 63	82. 59
	40	cer.ele.can	Cervus elaphus canadensis*	AB02109616	93, 63	90, 61
	41	cer.nip.ce	Cervus nippon centralis	AB02109415	98, 63	90, 61
	42	cer.nip.ye	Cervus nippon yesoensis	AB021095'*	98, 63	90, 61
	43	cer.nip.ke	Cervus nippon keramae	AB02109115	98, 63	90, 61

				49	00.40
44	cer.nip.pu	Cervus nippon pulchellus	AB02109016	98, 63	90, 61
45	cer.nip.ni	Cervus nippon nippon	AB02109314	98, 63	90, 61
46	cer.ela.sc	Cervus elaphus scoticus	AB02109916	98, 63	90, 61
47	cer.dam	Cervus dama	AJ00002214	98, 63	88, 53
43	ran.tar	Rangifer tarandus	AJ00002914	98, 63	89, 57
49		Moschus fuscus®	AF026888'7	90, 59	90, 61
50		Moschus leucogaster*	AF02688917	90, 59	90,61
51	mos.chr	Moschus chrysogaster*	AF026887 ¹⁷	90, 59	90, 61
52	mos.ber	Moschus berezovskii *	AF02688617	90, 59	90,61
53		Moschus moschiferus *	AF02688317	90, 59	92, 61
54	kob.ell	Kobus ellipsiprymnus	AF0220593	91,61	95, 66
55	kob.meg	Kobus megaceros	AJ222686 ¹	91, 61	83, 56
56	_	Redunca arundinum	AF09662313	91,61	94, 62
	red.ful	Redunca fulvorujula	AF0362841	89, 57	94, 62
58		Neotragus moschatus	AJ2226831	89, 57	94, 62
59	pel.cap	Pelea capreolus	AF0220551	91,61	90, 61
60	anteer	Antilope cervicapra*	AF022058 ³	82, 56	93, 64
61	sai.tat	Saiga tatarica	AF06448718	91,61	92, 61
62	gaz.dam	Gazella dama	AF0259543	91,61	92, 61
63	our.our	Ourebia ourebi	AF0362881	82, 56	82, 59
64	gaz.gaz	Gazela gazella •	AJ2226821	91,61	89, 57
65	rap.mel	Raphicerus melanotis	AF0220533	81, 54	80, 50
66	mad.kir	Madoqua kirkii	AF0220703	90, 58	97, 65
67	antame	Antilocapra americana	AF0916296	98, 63	98, 68
68	tra.jav	Tragulus javanicus*	D3218919	86, 57	86, 59
69	талар	Tragulus napu*	X56288 ³⁰	81, 52	93, 58
70	bal.acu	Balaenoptera acutorostrata	X75753 ²¹	89, 56	97, 61
71	bal.bon	Balaenoptera bonaerensis	X7558121	89, 56	93, 59
72	bal.bor	Balaenoptera borealis*	X75582 ²¹	89, 56	93, 59
73	bal.edi	Balaenoptera edeni	X75583 ²¹	89, 56	83, 54
74	esc.rob	Eschrichtius robustus *	X75585 ²¹	97, 61	86, 57
75	bal.mus	Balaenoptera musculus*	NC_00160122	97, 57	93, <i>5</i> 9 94, 63
76	meg.nov	Megaptera novaeangliae •	X75584 ²¹	97, 61	94, 63
77	bal.phy	Balaenoptera physalus*	NC_001321 ²³	97, 57 93, 55	91, 53
	cap.mar	Caperea marginata	X75586 ²¹ AF084073 ²⁴	85, 51	38, 55
79	cep.com	Cephalorhynchus commersonii	AF084073	85, 51	92, 59
	cep.cut	Cephalorhynchus eutropia*	AF084072 AF084067 ²⁴	94, 59	92, 59
81	lag.obi	Lagenorhynchus obliquidens Cephalorhynchus heavisidii	AF084077	89, 56	97, 63
	cep.hea	cephalorhynchus hectori	AF08407124	89, 56	92, 59
	lag.aus	Lagenorhynchus australis	AF084069 ²⁴	86. 54	92, 59
	lag.cru	Lagenorhynchus cruciger	AF08406824	86.54	92, 59
	lag.obs	Lagenarhynchus obscurus	AF084066:4	86. 54	92, 59
	lis.bor	Lissodelphis borealis	AF08406424	12,28	92. 59 °
	lis.per	Lissodelphis peronii	AF054065:4	86, 54	92, 59
89	glo.mac	Globicephala macrorlynchus	AF084055:4	94, 59	88, 55
90	glo.mel	Globicephala melas	AF084056:4	94, 59	88. 55
91	fer.att	Feresa attenuata*	AF084052 ²⁴	94. 59	92, 59

_	a tota doma	AF0840532*	94, 59	88, 55
92 pep.ele	Peponocephala electra*	AF084059 ²⁴	97,61	89, 59
93 gra.gri	Grampus griscus	AF084057	94, 59	92, 59
94 pse.cra	Pseudorca crassidens*	AF084075 ¹⁴	98, 63	89. 59
95 lag.acu	Lagenorhynchus acutus	AF08406124	86, 57	82, 52
96 orci.bre	Orcinus orca	AF084001	86, 57	91,54
97 orea.bre	Orcaella breviros:ris	AF084063 ²⁴	96, 54	97, 63
98 del.cap	Delphinus capensis	AF084087 ²⁴	97, 57	97, 63
99 del.tro	Delphinus tropicalis	AF08408S ²⁴	97, 57	97, 63
100 del.del	Delphinus delphis	AF084085 ³⁴	97, 57	97, 63
101 sten.cly	Stenella clymene	AF084083 ²⁴	97, 57	97, 66
102 sten.coe	Stenella coeruleociba	AF084082 ²⁴	97, 57	97, 63
103 tur.adu	Tursiops aduncus	AF084092 ²⁴	97, 57	97, 63
104 sten.fro	Stenella frontalis	AF084090 ²⁴	97, 57	88, 59
105 saus.chi	Sousa chinensis	AF084080 ²⁴	97, 57 97, 61	97, 63
106 sten.lon	Stenella longirostris	AF084103 ²⁴	97, 57	96, 59
107 turs.tru	Tursiops truncatus	AF08409524	97, 57 97, 61	97, 66
108 lage.alb	Lagenorhynchus alborostris	AF084074 ²⁴	97, 61	94, 64
109 sten.bre	Steno bredanensis	AF084077 ²⁴	97, 61 97, 61	97, 63
110 sota.flu	Sotalia fluviatilis	AF304067 ²⁵	97, 61 97, 61	95, 66
111 del.leu	Delphinapterus leucas	U72037 ²⁶	97, 61	95, 66
112 mono.mon	Monodon monoceros	U72038 ²⁶	97, 61	86, 59
113 plat.gan	Platanista gangetica*	AF304070 ²⁵	97, 61	86, 59
114 platmin	Platanista minor*	X92543 ²⁷	97, 51	90, 63
115 kogi.bre	Kogia breviceps	U72040 ²⁵	96, 55	92, 63
116 kogi.sim	Kogia simus	AF304072 ³³ AF304073 ²⁵	97, 57	80, 58
117 phys.cat	Physeter catodon	AF304071 ²⁵	89, 56	83, 53
118 lipo.vex	Lipotes vexillifer*	AF084051 ²⁴	87, 49	92, 62
119 phoc.sin	ркосоела зіпиз	X92541 ²⁷	96, 55	90, 59
120 bera bai	Berardius bairdii	X92540 ²⁷	97,61	89, 57
121 ziph.car	Ziphius cavirostris	X92537 ²⁷	97, 57	90, 61
122 meso.eur	Mesoplodon europaeus	X92538 ²⁷	97, 61	92, 61
123 meso.bid	Mesoplodon bidens	X92536 ²⁷	91,61	94, 63
124 meso.den	Mesoplodon densirostris Hyperoodon ampullatus •	X92539 ²⁷	97,61	90,65
125 hype.amp	Mesoplodon peruvianus	AF304074 ²³	97, 61	86, 58
126 meso.per	Pontoporia blainvillei	AF304069 ²⁵	92, 59	88, 55
127 pontbla	Hippopotamus amphibius	Y08813 ²⁹	92, 53	95. 66
128 hipp.amp 129 hex.lib	Hexaprotodon liberiensis	Y0831429	98, 63	. 97, 66
130 rhin.son	Rhinoceros sondaicus*	AJ245725 ¹⁰	90, 59	37, 61
131 cera	Ceratotherium simum	NC 00130212	90, 59	90.63
132 dic.sum	Dicerorhinus sumatrensis	AJ24572310	90, 59	86. 57
t33 equu	Equus asinus	NC_001788"	91,61	73.51
134 baby.bab	Babyrousa babyrussa	Z50106"	89, 56	85, 56 87, 54
t35 phac.afr	Phacochocrus africanus	Z50090 ¹¹	90, 59 97, 57	83.54
136 sus.scr.ew	Sus scrofa haplotype EWB3*	AF136549 ¹⁴	97, 57 97, 57	85. 55
137 sus.bar	Sus barbatus	Z50107"	87, 55	85. 53
138 lama.gla	Luma glama	C0645311	83, 54	86. 57
t39 lama.gua	lama guanicae	Y08812 ²⁷	, -	-

			U06430 ³³	89, 55	85, 53
140 vic.vic	Vicugna vicugna		U06427 ³⁵	94, 58	86, 58
141 cam.bac	Camelus bactrianus		XS229316	97, 60	87, 64
142 arc.for	Arctocephalus forsteri		X82292 ¹⁶	94, 58	87,64
143 arc.gaz	Arctocephalus gazella		X32192	97, 57	86, 57
144 cum.jub	Eumetopias jubatus		X32311 ¹⁶	89, 55	86, 57
145 zal.cal	Zalophus californianus		XS2310 ³⁶	91.61	81,52
146 odo.ros	Odobenus rosmarus		X3229936	90, 58	87, 64
147 pho.vit	Phoca vitulina		X32306 ¹⁶	98, 63	95, 66
148 pho.fascia	Phoca fasciata		XS230236	93, 63 92, 59	90, 61
149 pho.gro	Phoca groenlandica		X82303 ¹⁶	89, 56	87, 64
150 cys.cri	Cystophora cristata		X82294 ³⁶		82, 54
151 hyd.lep	Hydrurga leptonyx	:	X3229736	89, 55	91,66
152 lep.wed	Leptonychotes wedde!li		X72005 ³⁷	98, 63	82, 59
153 mir.leo	Mirounga leonina		X32298 ³⁶	89, 55	87, 63
154 eri.bar	Erignathus barbatus		X82295 ³⁶	89,56	87, 60
155 mon.sch	Monachus schauinslandi		X72209 ³⁷	91,61	90, 63
156 hela mal	Helarcios malayanus *		U1889938	84, 54	87, 64
157 sel.thi	Selenarctos thibetanus		AB02091039	89, 57	87,64
158 ail.ful	Ailurus fulgen*s		X94919 ⁴⁶	93, 55	90, 63
159 fel	Felis catus		NC_00170041	85, 56	84, 54
160 can	Canis familiaris		NC_00200812	98, 58	92, 57
161 tal	Talpa europaea		NC_00239143	81,50	82, 54
162 gla.sab	Glaucomys sabrinus		AF01173844	90, 59	87, 60
163 gla.vol	Glaucomys volans		AB03026145	90, 59 91, 61	81,50
164 hyl.pha	Hylopetes phayrei*		AB03025945	91,61	81,50
165 pet.set	Petinomys setosus*		AB03026045	91,61	87, 64
166 bel.pea	Belomys pearsonii*		AB03026245	97, 61	90, 63
167 ptc.mom	Pteromys momonga*		AB030263*5	97, 58	87, 6 4
168 gala.demi	Galagoides demidojj		AF27141146	97,60	87, 63
169 pero.pot	Perodicticus potto		AF2714134	97, 60	90, 61
170 gala.mat	Galago matschiei		AF2714094	97, 57	95, 66
171 gala.moh	Galago moholi		AF271410 ⁴⁴ AF271412 ⁴⁴	92, 58	87, 60
172 oto.gar	Otolemur garnettii		U5358147	97,60	93. 59
173 lor.tar	Loris tardigradus*		US3580 ⁴⁷	97, 60	95, 66
174 nyc.cou	Nycticebus coucang*		NC_0015694	97,60	86, 59
175 mus	Mus musculus		MC_001942	89, 57	80, 58
176 gorr	Gorilla gorilla		NC_00180719	96.55	84.64
177 homo	Homo sapiens sapiens		U0756451	97.60	89, 59
178 dug.dug	Dugong dugong *		AB00241212	97, 60	76.57
179 ele.max 130 afr.con	Elephas maximus* Afropavo congensis		AF013760"	97, 58	87, 63
181 pavo.mut	Pava mulicus*	1	AF013763"	97, 57	87, 63
182 tra.bly	Tragopan blythii*		AF200722"	89. 55	85, 57
183 tra.sat	Тгадоран загуга*		AF22983734	89.55	86.61
184 tra.cob	Tragnpan caboti		AF20072314	89, 55	\$6.61
185 tra.tem	Tragopan temmineku*		AF028502"	87.55	81.56 87.63
186 arg.arg	Argusianus argus		AF013761"	89, 55	87, 63 85, 57
187 cat.wal	Catrous wallichi*		AF028792"	33, 54	43, 3.

•		. 500.070.433	89, 55	85, 57
188 cro.cro	Crossoptilon crossoptilon •	AF028794 ⁵³	89, 55	85, 57
189 sym.ree	Syrmaticus reevesi*	AF028801 13	•	94, 64
190 bam.tho	Bambusicola thoracica*	AF02879053	80, 48	
191 fra.fra	Francolinus francolinus	AF013762 ³³	97, 58	86, 61
192 ith.cru	Ithaginis cruentus*	AF068193 ⁵³	98, 63	85, 57
193 ant.par	Anthropoides paradisca	U27557 ³⁶	85, 56	82, 58
194 ant.vir	Anthropoides virgo	U27545 ⁵⁶	84, 54	82, 52
195 gru.ant.an	Grus antigone antigone	U11060 ⁵⁷	90, 53	87, 63
196 gru.ant.gi	Grus antigone gillae	U11064 ⁵⁷	90, 58	87, 63
197 gru.any.sh	Grus antigone sharpei	U11061 ⁵⁷	90, 58	87, 63
198 gru.leu	Grus leucogeranus*	U27549 ⁵⁶	90, 53	87, 63
199 gru.can.pr	Grus canadensis pratensis	U27553 ⁵⁶	97, 60	87, 63
200 gru.can.ro	Grus canadensis rowani	U27552 ⁵⁶	97, 60	87, 63
201 gru.can.ta	Grus canadensis tabida	U27551 56	98, 63	87.63
202 gru.can.ca	Grus canadensis canadensis	U27554 ⁵⁶	97, 61	87, 63
203 gru.ame	Grus americana	U27555 ⁵⁶	90, 58	87, 63
204 gru.gru	Grus grus	U27546 ⁵⁶	89, 54	87, 63
205 gru.mon	Grus monacha *	U27548 ⁵⁶	90, 53	87, 63
206 gru.nig	Grus nigricollis®	U27547 ⁵⁶	90, 58	87, 63
200 gru.jap	Grus japonensis	U27550 ³⁶	81,54	87, 63
207 glu.jap 208 cic.boy	Ciconia boyciana*	NC_002196 ⁵⁸	94, 58	79, 60
209 the.ame	Rhea americana	AF09033959	93, 63	79, 60
210 antalb	Anthracoceros albirostris*	U89190 ⁶⁰	97,61	86, 59
210 ancaio 211 fal.fam	Falco femoralis	U83310 ⁶¹	97, 61	86, 60
212 fal.ver	Falco verpertinus	U83311 ⁶¹	97,61	85, 57
213 fal.par	Falco peregrinus •	U83307 ⁶¹	97, 61	84, 52
214 fal.spa	Falco sparverius	U83306 ⁴¹	92. 59	80, 51
215 aytame	Aythya americana	NC_000877 ⁶²	98, 63	94, 62
216 smi.sha	Smithornis sharpei	NC_00087959	97, 58	90,61
217 vid.cha	Vidua chalybeata	NC_000830 ₂₆	97, 60	87, 64
218 chry.pic	Chrysemys picta	NC_002073 ⁶³	39, 56	86, 57
219 emy.orb.ku	Emys orbicularis	AJ131425 ⁶⁴	90, 59	94, 63
220 che.mud	Chelonia mydas °	AB012104 ⁶⁵	90, 58	94, 63
221 eum.egr	Eumeces egregius	AB016606*5	86. 55	73, 51
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WO 02/077278 28 PCT/IN01/00055

Table 2. Multiple sequence alignment of 472 bp fregment of mitochondrial cytochrome b gene of 221 animal species

PRIMER 'mcb393'	TACCATGAGGACAAATATGATTGTG	
PRIMER MCC3336		
1		6 :}
aep.mel		50
012.012		3.7
add.nas	TO CONTROL CONTROL TO THE TOTAL ACCOUNTATION OF THE CONTRACT O	50
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cep.max		
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pos.gru		
bos.tra		
bub.min		
buba.bub		-
tra.ang	TOCCATGAGGACAACIATCATCTGAGGAGGAACGGGTGATCACAAACCTCCTATGAGGAA TOCCATGAGGACAACTATGATTCTGAGGAGGAACGGGTGATCACAAACCTCCTATGAGGAA	63
tra.eur	TOCCATGAGGACAATATGATTCTGAGGAGCAACAGTCATCACAAACCTTCTATCAGCAA TACGATGAGGACAAATATGATTTTGAGGAGCAACAGTCATCACAAACCTTCTATCAGCAA	60
kob.ell	TACCATGAGGACAAATATCCTTCTGAGGAGCAACAGTCATCACCAATCTCCTTTCAGCAA TACCATGAGGACAAATATCCTTCTGAGGAGCAACAGTCATCACTAATCTCCTTTCAGCAA TACCATGAGGACAAATATCCTTCTGAGGAGCGACAGTCATCACTAATCTCCTTTCAGCA	50
kob.meg	TACCATGAGGACAATATCCTTCTGAGGAGCAACAGTTATCACTAATCTTCTCTCAGCAA TACCATGAGGACAAATATCCTTCTGAGGAGCAACAGTTATCACTAATCTTCTCTCAGCAA	60
red.aru	TACCATGAGGACAAATATGCTTCTGAGGAGCAACAGTTATGACTTAACGTTCTCTCAGCAA	60
red.ful		
neo.mos		50
pel.cap		-
gaz.dam our.our		
ami.Ct:		-
sai.tat		
mai.ki:		
sas.mel		
gaz.gaz		
ant.ame	AADDADTOTECTEATOATATO ATTACTC AC AADDOOCG ACTETTACTAACGATCACDATAACGATCACDATAACGATCACDATAACGATCACDATAACGATCACDATAACGATCACDATAACAACACACACACACACACACACACACACAC	ร์ง
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cer.ela.xan	TACCATGAGGACAAATATCATTCTGAGGAGCAACGGTCATTACCAACCTTCTCTCAGCA	
cer.ela.can	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTTCTCTCAGCA	
cer.nip.cent	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTCCTCTCAGCA	
cer.nip.yes	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTCCTCTCAGCAA	
cer.nip.ker	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTTCTCTCAGCA	
cer.nip.pul	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTTCTCTCAGCAA	
cer.nip.nip	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACCAACCTCCTCTCAGCAA	
cer.ela.sco	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTTCTCTCAGCAA	
cer.dam	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTTATTACCAATCTTCTCTCAGCAA	
ran.tar	TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTTATCACAAACCTCCTCTCAGCAA	
mos.fus	TACCTTGAGGACAAATATCTTTCTGAGGAGCGACAGTTATTACCAATCTTCTCTCAGCAA	
mos.leu	TACCTTGAGGACAAATATCTTTCTGAGGAGCAACAGTTATTACCAATCTTCTCTCAGCAA	
mos.chr	TACCTTGAGGACAAATATCTTTCTGAGGAGCAACAGTTATTACCAATCTTCTCTGAGGAA	
mos.ber	TACCTTGAGGACAAATATCTTTCTGAGGAGCAACAGTTATTACCAATCTTCTCTCAGCAA	
mos.mos	TACCTTGAGGACAATATCTTTCTGAGGAGCAACAGTCATCACTAACCTTCTCTCAGCAA	
tra.jav	TACCCTGAGGACAGATATCTTTCTGAGGAGCCACAGTCATCACCAACCTCTTATCAGCTA	. 60
trag.nap	TACCCTGAGGGCAAATATCTTTTTGAGGAGCTACAGTCATCACCTAACCTTCTTTCAGCAA	. 60
bala.acu	TACCCTGAGGACAATATCATTTTGAGGTGCAACCGTCATCACCAACCTCCTATCAGCAA	. 60
bala.bon	TACCCTGAGGACAAATATCATTTTGAGGCGCAACCGTCATCACCAACCTCCTATCAGCAA	. 60
bala.bor	TACCCTGAGGACAATATCATTTTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	60
bala.edi	TACCCTGAGGACAATATCATTTTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	60
esch.rob	TACCCTGAGGACAAATATCATTCTGAGGCGCAACCGTTATCACCAACCTCCTATCAGCAA	60
bala.mus	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCCTATCAGCAA	60
mega.nov	TACCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTTCTATCAGCAA	60
bala.phy	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACTGTAATCACTAACCTCCTATCAGCAA	60
cap.mar	TGCCCTGAGGACAGATATCATTCTGAGGCGCAACCGTCATCACCAACCTCCTATCAGCAA	60
ceph.com	TACCCTGGGGACAGATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA	60
ceph.eut	TACCCTGGGGACAGATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA	60
lage.obl	TACCCTGAGGACAGATATCATTCTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA	60
ceph.hea	TACCCTGAGGACAAATATCATTTTGAGGCGCAACAGTCATCACCAACCTCCTATCAGCAA	60
ceph.hec	TACCCTGAGGACAAATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA	60
lage.aus	TACCCTGAGGACAGATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA	60
lage.cru	TACCCTGAGGACAGATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA	60
lage.obs	TACCCTGAGGACAGATATCATTTTGAGGTGCAACAGTCATCACCAACCTCCTATCAGCAA	60
lisso.bor	TACCCTGAGGGCAGATATCATTTTGAGGTGCAACCGTCATCACCAACCTCCTATCAGCAA	60
lisso.per	TACCCTGAGGACAGATATCATTTTGAGGTGCAACCGTCATCACCAACCTCCTATCAGCAA	60
glo.mac	TACCCTGAGGACAGATATCATTCTGAGGGGCAACCGTCATCACCAATCTCCTATCAGCAA	60
glo.mel	TACCCTGAGGACAGATATCATTCTGAGGGGCAACCGTCATCACCAATCTCCTATCAGCAA	60
fere.att	TACCCTGAGGACAGATATCATTCTGAGGGGCAACCGTCATCACCAATCTCCTATCAGCAA	60
pepo.ele	TACCCTGAGGACAGATATCATTCTGAGGGGGCAACCGTCATCACCAATCTCCTATCAGCAA	60
gram.gri	TACCCTGAGGACAAATATCATTCTGAGGGGGCAACCGTCATCACCAATCTCCTATCAGCAA	60
pse.cra	TACCCTGAGGACAGATATCATTCTGAGGGGGCAACCGTCATCACCAATCTTCTATCAGCAA	60
lage.acu	TACCATGAGGACAAATATCATTCTGAGGGGGCAACCGTTATCACCAATCTCCTATCAGCAA	£0
ordi.bre	TACCCTGAGGACAGATATCTTTCTGAGGGGCAACCGTCATTACTAATCTCCTATCAGCAA	60
crca.bre	TACCCTGAGGACAGATATCCTTCTGAGGTGCAACCGTCATCACCAATCTCCTATCAGCAA	60
del.cap	TGCCCTGGGGACAATATCATTCTGAGGGGGCAACCGTCATCACCAACCTCTTATCAGCAA	60
del.tro	TGCCCTGAGGACAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	= 0
del.del	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	60
sten.cly	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCCTATCAGCAA	60
sten.coe	TGCCCTGAGGACAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	50
tur.adu	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	60
sten.fro	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	50
saus.chi	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTTATCACCAACCTCCTATCAGCAA	60
stem.lon	TACCCTGAGGACAAATATCATTCTGAGGTGCAACCGTCATCACCAACCTCCTATCAGCAA	60
turs.tru	TGCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAACCTCTTATCAGCAA	6 O
lage.alb	TACCCTGAGGACAAATATCATTGTGAGGCGCAACCGTCATCACTAATCTGCTATCAGCAA	2 O
sten.bre	TACCCTGAGGACAAATATCATTCTGAGGTGCAACCGTCATTACCAACCTCCTGTCAGCAA	6 U
sota.flu	TACCCTGAGGACAAATATCATTCTGAGGGGCAACCGTCATTACCAATCTCCTATCAGCAA	9 4

		63
del.leu	TACCCTGAGGACAAATATCATTCTGAGGGGGCAACCGTCATTACCAATCTCCTATCAGCAA	63
mono.mon	TACCCTGAGGACAAATATCATTCTGAGGTGCAACCGTCATCACCAACCTCCTATCAGCAA	60
plac.gan	TACCCTGAGGACAAATATCATTCTGAGGTGCAACCGTCATCACCAACCTTTTTATCAGCAA	60
plat.min	TACCCTGAGGACAAATATCATTCTGAGGTGCAACCGTCATCACCAACCTTTTATCAGCAA	60
kogi.bre	TACCCTGAGGCCAAATATCATTCTGAGGAGCAACCGTCATCACCAACCTTATATCCGCAA	63
kogi.sim	TGCCCTGAGGCCAAATATCATTCTGAGGAGCAACCGTCATCACAAACCTTATATCCGCAA	5.3
phys.cat	TGCCCTGAGGACAAATATCATTCTGAGCCGCAACCGTTATCACAAACCTTCTATCAGCAA	6.)
lipo.vex	TACCCTGAGGACAAATATCATTTTGAGGCGCAACCGTCATCACTAATCTTCTATCAGCAA	63
phoc.sin	TGCCCTGGGGACAAATATCATTTTGAGGTGCTACCGTCATCACAAACCTCTTATCAGCAA	60
bera.bai	TGCCTTGAGGGCAAATATCATTCTGAGGTGCAACCGTCATCACCAACCTCCTATCCGCTA	60
ziph.car	TACCTTGAGGACAAATATCATTCTGAGGTGCAACCGTCATCACAAACCTCTTATCCGCTA	60
meso.eur	TTCCCTGAGGACAAATATCATTCTGAGGCGCAACCGTTATTACCAACCTCCTATCCGCCA	6.0
meso.bid	TACCCTGAGGACAAATATCATTCTGAGGGGGCAACTGTTATTACTAACCTCCTATCCGCTA	60
meso.dem	TACCATGAGGACAAATATCCTTCTGAGGTGCAACTGTCATTACCAATCTTCTATCCGCTA	60
hype.amp	TACCCTGAGGACAAATATCATTCTGAGGCGCAACCGTCATCACCAATCTCCTATCCGCCA	60
meso.per	TACCTTGAGGACAAATATCATTCTGAGGCGCAACTGTCATTACTAATCTTTTATCTGCTA	60
pont.bla	TACCCTGAGGACAAATGTCATTCTGAGGTGCCACTGTCATCACCTAACCTCCTATCAGCGA	60
hex.lib	TACCATGAGGACAAATATCATTCTGAGGGGGCAACAGTCATCACCAACTTACTATCAGCTA	63
hipp.amp	TGCCATGAGGACAAATGTCATTCTGAGGGGCAACAGTCATTACCAACTTACTGTCAGCTA	60
dic.sum	TACCATGAGGTCAAATATCCTTCTGAGGAGCCACAGTTATCACAAATCTCCTCTCAGCCA	60
rhin.son	TACCATGAGGTCAAATATCCTTCTGAGGGGCTACAGTCATTACAAATCTCCTCTCAGCCA TACCATGAGGCCAAATATCCTTCTGAGGGGCTACAGTCATCACAAACCTCCTCTCAGCTA	60
cera	TACCATGAGGCCAAATATCCTTCTGAGGGGCTACAGTCACCACAAACCTCCTATCAGCAA TACCATGAGGACAAATATCCTTCTGAGGAGCAACGGTCATTACAAACCTCCTATCAGCAA	60
edra	TACCATGAGGACAAATATCCTTCTGAGGAGCAACGGTCATTACAAACCTACTATCAGCCA TACCTTGAGGACAAATATCATTTTGAGGAGCTACCGTCATTACAAACCTACTATCAGCCA	60
baby.bab	TACCTTGAGGACAATATCATTTGAGGAGCACAGTCATCACAAACCTACTATCAGGCA	60
phac.afr	TGCCTGAGGACAATATCGTTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGGTA	60
sus.bar	TGCCCTGAGGACAAATATCATTCTGAGGAGCTACGGTCATCACAAATCTACTATCAGCTA	60
sus.scr.ewb3	TCCCATGAGGACAAATATCATTTTGAGGGGCAACAGTAATTACAAATCTACTCTCGGCAA	60
lama.gla	TCCCATGAGGCCAAATATCATTTTGAGGGGCAACAGTAATTACAAACCTACTCTCGGCAA	60
lama.gua vic.vic	TCCC1TG1GG1C111TTTG1GGGGGCAACAGTAATTACAAACCTACTCTCAGCAA	60
cam.bac	TOCOLTOLOGICACIA TATOLTTOTOGGGAGCAACAGTAATTACCAACCTACTCTCAGCAA	60
arc.for	TTCLTGLGGLCLLATTCLTTCTGAGGAGCGACCGTCATTACCAACCTCCTATCAGCAG	5 U
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glo.mac	ATTTCCTATTCGCATAGCAATCTTACGATCAATTCCCAATAAACTTGGAGG ATTTCCTATTCGCATATGCAATCTTACGATCAATTCCCAATAAACTTGGAGG	472
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sca.flu	ATTTCCTATTCGCATACGCAATCTTACGATGAATGGCTAATAAACTTGGAGG ATTTCCTATTCGCATATGCAATCTTACGATGAATGGAGGAACTAGGAGG	472
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glas.gan	ATTTCCTATTTCCATACGCAATTCTACGATAAACTAGGAGG ATTTCCTATTTCCATACGCAATTTTACGGTCAATACCTAATAAACTAGGAGG	477
plac.min	ATTTECTATTTECATACECAATTTTACEGTTAATCTACAATAAACTAGGGGG	477
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cer.ela.xan	CACACAACTATACCCCAGCAAATCCACTCAACACACCCCCTCACATTAAACCAGAAAAAA	476
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cer.mip.mip	CAGATAACTACACCCCAGCAAACCCCACTCAACACACCCCCTCATATTAAACCTGAATGAT	420:
cer.ela.sco	CAGACAAATACACTGCAGCAAATGCACTCAACACACCTCGTCATATTAAACGCGGAATGAT	420
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ran.tar	CGGACAATTATACCCCAGCAAACCCATTAAATACGCCCCACATATTAAACCCGAATGAT	420
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mon.sch	ACTTCCTATTCGCCTACGCATCCTACGATCCATCCCTAATAAACTAGGAGG ACTTTCTATTTGCCTACGCTATCCTACGATCCATCCCTAAAACTAGGAGG	472
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sel.thi	ACTITITATITGCTTACGCTATCCTACGATCCATACCAAACAAACTAGGAGG ATTTCCTATTCGCATATGCAATTCTACGATCCAAACAAAC	472
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can tal	ATTITCTATICGCTATGCTATGCTACGATCATICCTAATAAATTAGGAGG ACTICCTATITGCATATGCCATCCTACGATCAATTCCTAATAAATTAGGAGG	472
gla.sab	ACTTECTATITECATATECCATCCTACGATCTATTCCAAATAAACTAGGAGG ACTTTCTATTTCCATACGCAATTCTACGATCTATTCCAAATAAACTAGGAGG	472
gla.vol	ACTITICIATITICATACGAATTCTACGATCTATCCCAAATAAACTAGGAGG ACTITICTATITICCGTATGCAATTCTACGATCTATCCCAATAAATTAGGAGG	472
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pie.mom	ATTTCCTATTCCCATAIGCIAICLIACGATTATACTAGGAGG	472
gala.demi	ATTTCCTATTTGCCTACGCCATCCTACGATCTACGATCTAGGGAGG	472
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Reference:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: 984593689-1224-27770 |

Query-

(328 letters)

Database: Sequences from complete mitochondrial genomes 129 sequences; 3,164,247 total letters

If you have any problems or questions with the results of this search please refer to the BLAST FAQS

Taxonomy reports

Distribution of 80 Blast Hits on the Ouerv Sequence

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(bits) Value Sequences producing significant alignments: 365 e-101 ref[NC_001700.1] Felia catus mitochondrion, complete genome 198 le-51 Phoca vitulina misochondrion, complete genome ref[NC_001325.1] 190 4e-49 Canis familiaris mitochondrion, complete g... ref NC 002008.1 180 Je-46 Halichoerus grypus mitochondrion, complete... T-EINC 001602.1 176 Se-45 Cavia porcellus complete mitochondrial genome ref[NC_000884.1] 155 2e-41 Ceracotherium simum mitochondrion, complet... ref NC 001808.1 153 8e-38 Myoxus glis mitochondrion, complete genome ref NC 001892.1 Equus asinus mitochondrion, complete genome 151 Je-37 ref NC 001783.1 Orycteropus afer complete mitochondrial ge... 149 le-36 TEE NC 002073.11 141 Je-34 Dasypus novemcinctus mitochondrion, comple... TeSINC 001821.1 R.unicornis complete mitochondrial genome 135 2e-32 Te NC 001779.1 133 125 7e-32 Mus musculus mitochondrion, complete genome ref[NC 001569.1] Hippoporamus amphibius mitochondzion, comp... 2e-29 125 2e-29 Equus caballus mitochondrion, complete genome ref[NC 001640.1] 123 7e-29 Macropus robustus mitochondrion, complete ... == [NC 001794 1] ref[NC 000845.1] Sus scrofa mitochondrion, complete genome 121 Je-28 121 3e-28 Rattus norvegicus mitochondrial genome ref NC 001665.1 121 3e-28 117 4e-27 Bos taurus mitochondrion, complete genome ref NC 001567.1 Pan troglodytes mitochondrion, complete ge... 109 le-24 Ovis aries mitochondrion, complete genome ref NC 001941.1 Talpa europaea mitochondrion, complete genome 103 7e-23 ref[NC 002391.1] 103 7e-23 101 3e-22 98 4e-21 Oryccolagus cuniculus mitochondrion, compl... ref[NC 001913.1] Pan paniscus mitochondrion, complete genome Tef NC 001644.1 Human mitochondrion, complete genome ref[NC 001807.2] 98 4e-21 Balaenoptera musculus mitochondrion, compl... resINC 001601.11 ref[NC 002009.1] Artibeus jamaicensis mitochondrion, comple... <u>95</u> 2e-20 92 Ze-19 ref NC 001645.1| Gorilla gorilla mitochondrion, complete ge... <u>90</u> le-18 Balaenoptera physalus mitochondrion, compl... ref NC 001321.11 80 9e-16 Didelphis virginiana mitochondrion, comple... ref NC 001610.1| ref NC 002082.1| 70 9e-13 70 9e-13 Hylobates lar mitochendrien, complete genome Crossostoma lacustre mitochondrion, comple... refiNC 001727.1 68 4e-12 56 1e-11 64 6e-11 Latimeria chalumnae mitochondrion, complet... Tet NC 001804.1 Vidua chalybeata mitochondrion, complete g... TO 1 NC 000880.1 Corvus frugilegus mitochondrion, complete ... == FINC 002069.11 <u>62</u> 2e-10 Chelonia mydas mitochondrial DNA, complete... Tef NC 000886.11 62 2e-10 62 2e-10 60 9e-10 Pongo pygmaeus mitochondrion, complete genome Tef NC 001646.1 Cyprinus carpio mitochondrion, complete ge...
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Rhea americana mitochondrion, complete genome <u>56</u> le-08 _54 Se-08 Ciconia boyciana mitochondrion, complete g... Te: | NC 002196.1 54 Se-08 54 Se-08 52 2e-07 Salmo salar mitochondrion, complete genome == [NC 001960.1] Polypterus ornatipinnis mitochondrion, com... == [NC 001778.1] Pongo pygmaeus abelii mitochondrion, compl... FEE NC 002083.1 52 2e-07 Struthio camelus complete mitochondrial ge... == [NC 001953.1] 52 2e-07 52 2e-07 TESING 001947.11 Pelomedusa subruta mitochondrion, complete... 2e-07 refinc 001770.11 Arbacia lixula mitochondrion, complete genome Alignments tgaatetgaggaggetteteagtagaeaaagetaeestgaeaegattetttgestteeae 60 ರಜ್ಞಾತಕದ್ದ0 1 NC 001892 14654 ... C. ... C. ... C. ... C. ... C. ... 14713 NC 001789 14671 ... C. ...

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Database: Sequences from complete mitochondrial genomes Posted date: Jun 28, 2000 10:56 AM Number of letters in database: 3,164,247 Number of sequences in database: 129

Lambda K H 1.37 0.711 1.31

Gapped
Lambda K H
1.37 0.711 1.31

Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Rits to DB: 788
Number of Sequences: 129
Number of extensions: 788
Number of successful extensions: 168
Number of sequences better than 10.0: 77
length of query: 328
length of database: 3,164,247
effective HSP length: 15
effective length of query: 313
effective length of database: 3,162,312
effective search space: 989803656
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A: 30 X1: 6 (11.9 bits) X2: 15 (29.7 bits) S1: 12 (24.3 bits) S2: 14 (28.2 bits)

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BLASTN 2.1.2 [Nov-13-2000]

Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997).
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.

RID: 984593400-28182-3122

Query-

(328 letters)

Databasa: nt

807,597 sequences; 2,863,827,885 total letters

If you have any problems or questions with the results of this search please refer to the \underline{BLAST} \underline{FAQs}

Taxonomy reports

Distribution of 50 Blast Hits on the Ouery Sequence

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Database: nt

Posted date: Mar 2, 2001 12:20 AM Number of letters in database: 2,861,827,885 Number of sequences in database: 807,597

Lambda 0.711 1.31 1.37

Gapped Lambda 0.711 1.37

Matrix: blastn matrix:1 -3 Gap Penalties: Existence: 5, Extension: 2 Number of Hits to DB: 460542 Number of Sequences: 807597 Number of extensions: 460542 Number of successful extensions: 22671 Number of sequences better than 10.0: 6487 length of query: 328 length of database: 2,863,827,885 effective RSP length: 20 effective length of query: 308 effective length of database: 2,847,675,945 effective search space: 877084191060 effective search space used: 877084191060 T: 0 A: 30 X1: 6 (11.9 bits) X2: 15 (29.7 bits) S1: 12 (24.3 bits)

S2: 19 (38.2 bits)

Table 5. Reference animals and the allocated code numbers included in the study

Sh	i. Code number	Name of the animal	Zoological name
1	bhz25t	Indian liger	Panthera tigris tigris
2	bhz26t	Indian tiger	Panthera tigris tigris
3	bhz30t	Indian tiger	Panthera tigris tigris
4	bhz45t	Indian tiger	Panthera tigris tigris
5	bhz56t	Indian tiger	Panthera tigris tigris
6	bhz63t	Indian tiger	Panthera tigris tigris
7	bhz20wt	Indian white tiger	Panthera tigris bengalensis
8	bhz22wt	Indian white tiger	Panthera tigris bengalensis
9	bhz23wt	Indian white tiger	Panthera tigris bengalensis
10	bhz28wt	Indian white tiger	Panthera tigris bengalensis
11	gz1l	Normal leopard	Panthera pardus
12	gz21	Normal leopard	Panthera pardus
13	gz3i	Normal leopard	Panthera pardus
14	gz21cl	Clouded leopard	Neofelis nebulosa
15	gz22cl	Clouded leopard	Neofelis nebulosa
16	darz14si	Snow leopard	Panthera unicia
17	darz15si	Snow leopard	Panthera unicia
18	darz16sl	Snow leopard	Panthera unicia
19	sbz22al	Asiatic lion	Panthera leo persica
20	sbz38al	Asiatic lion	Panthera leo persica
21	sbz39al	Asiatic lion	Panthera leo persica
22	humsk	Human	Homo sapiens sapiens
23	chimss	Chimpanzee	Pan sp.

Table 6. Multiple sequence alignments of the cytochrome b sequences of reference animals with the sequence obtained from confiscated animal remain

	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	50
sbz22al	TGAATCTGAGGAGGCTT. U.CAU. AUACAAAGCCACCCTTACACACCATTCTTTGCCTTCCAC	60
s5:38al	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
s5:39al	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
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bhz30t	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	63
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sbz22al	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAA	120
sbz38al	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAA TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAA	120
sbz39al	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAA TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCTCCATGAA	120
adil.flesh	TTCATCCTTCCATTTALCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCCTTCACGAG TTCATCCTTCCATTTATCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCCTTCACGAG	120
gzial	TTCATCCTTCCATTTATCATCTCACCTCTAGCAGCAGTCCACCTCCTATTCCTTCACGAG TTCATCCTTCCATTTATCATCTCACCTCTAGCAGCAGTCCACCTCCTATTCCTTCACGAG	120
gsinl	TTCATCCTTCCATTTATCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCCTTCACGAG TTCATCCTTCCATTTATCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCCTTCACGAG TTCATCCTTCCATTTATCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCCTTCC	120
gz3ml	TTCATCCTTCCATTTATCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG	120
bh=23wt	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGCTCCCACTCCTATTCCTCCATGAG TTCATCCTTCCATTTATCATCTCAGCCCCTAGCAGCAGCTCCCACTCCTATTCCTCCATGAG	120
bh:28wc	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG	120
bh=22vt	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG	120
bh:20wc	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCCATGAG	120
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gz2al	ACAGGATOTA ACA ACCOCTOAGGAATAGTATOTGACTCAGACAAAATTECATTCCACCCA	230
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bhz63t	ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA	180
bhz56t	ACAGGATOTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA	190
bh:26t	ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA	180
bhz30c	ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA	130
bhz45t	ACAGGATCTAACAACGCCTCAGGAATAGTATCTGACTCAGACAAAAATCCCGTTCCACCCA	130
bb:25t	ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA	120
d:14sl	ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCCACCCA	130
d:15sl	ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAAATCCCGTTCCACCCA	130
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gz21cl	ACAGGATCCAATAACCCCTCAGGAATGGTATCCGATTCAGACAAAATCCCGTTCCACCCG	180
gz22cl	ACAGGATCCAATAACCCCTCAGGAATGGTATCCGATTCAGACAAAATCCCGTTCCACCCG ACAGGATCAAATAACCCCCTGGGAATCACCTCCCACTCCGACAAAATTACCTTCCACCCC	130
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bh=25t	TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTCTACTACTACTACTA	240
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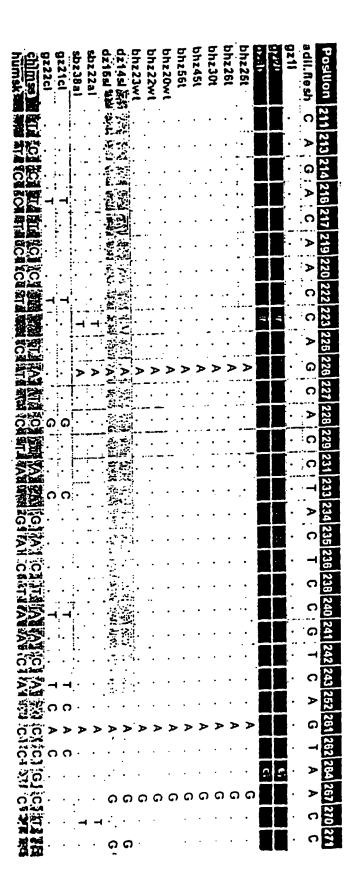
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bhz56t	GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGGCCAACCCTCTA 300
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gz21cl	GTTCTATTCTCCCCAGACCTACTAGGAGACCCTGACAATTACACTCCCGCCAACCCTCTA 300
gz22cl	GTTCTATTCTCCCCAGACCTACTAGGAGACCCTGACAATTACACTCCCGCCAACCCTCTA 300
chimss	ACACTATTCTCACCAGACCTCCTGGGCGATCCAGACAACTATACCCTAGCTAACCCCTA 300
humsk	ACACTATTCTCACCAGACCTCCTAGGCGACCCAGACAATTATACCCTAGCCAACCCCTTA 300
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gz2nl	AATACCCCTCCCCATATCAAGCCTGAAT 323
gzini	AATACCCCTCCCCATATCAAGCCTGAAT 328
bhz23wt	AACACCCCTCCCCATATCAAGCGCGAAT 328
bh=28wt	AACACCCCTCCCCATATCAAGCGCGAAT 329 **
bhz22wt	AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz20wt	AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz63c	AACACCCCTCCCCXTATCAAGCGCGAAT 323
bhz56t	AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz265	AACACCCCTCCCCATATCAAGCGCGAAT 328
bhz30t	AACACCCCTCCCCATATCAAGCGCGAAT 323
bhz45c	AACACCCCTCCCCATATCAAGCGCGAAT 329
bh=25t	AACACCCCTCCCCATATCAAGCGCGAAT 329
dz14sl	AACACCCCTCCCCATATCAAGCCCGAAT 328
dz15sl	AACACCCCTCCCCATATCAAGCCCGAAT 328
i=16sl	AACACCCCTCCCCATATCAAGCCCGAAT 329
g=21cl .	AATACCCCTCCCCATATCAAGCCTGAAT 328
3=22cl	AATACCCCTCCCCATATCAAGCCTGAAT 328
chimss	AACACCCCACCCACATTAAACCCGAAT 328
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Table 7b



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Table 7d

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bhz45t		•		:			· •	C			•	. • .	•	~	· • ·		•		. G	~
bhz56t						•		C	•	•	. •	•	•	~	•	• •.	: .	. •	G	~
bhz20wt	٠.			•	•			C		•	•	•	•	~	•	.*		•	G	
bhz22wt				• :		•	•	<u>ر</u>	•	•	•	•	•	Č	•	•	. •	•	9	<u> </u>
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humsk &	1CI	477							EG.		CY.	M.	S.1	C3		C		忍語	3 .3	(C]

Table 8. Percent similarity matrix calculated by pair-vise comparisons of cytochrome b gene sequences reveated from 'adil.flesh' and different felids

	bhz20wt bhz25	bhz25t	dz14si	humsk	chimss	sbz22al	Oz1L	gz2L	16z0	gz21ci	adil.flesh
bhz20w1		100	99.1	81.7	78.7	93.3	95.1	95.4	95.4	9.68	95.4
bhz25t	100		99.1	81.7	78.7	93.3	95.1	95.4	95.4	9.68	95.4
dz14sł	1.66	99.1		81.4	78.4	93	94.8	95.1	95.1	89.3	95.1
humsk	1.18	81.7	81.4		86.9	79.6	81.1	80.2	80.2	79	81.4
chimss	78.7	7.8.7	78.4	86.9		7.87	79.6	78.7	78.7	76.8	79.9
sbz22al	93.3	93.3	. 93	79.6	78.7		92.1	92.4	92.4	69	92.4
gz1L	95.1	95.1	94.8	81.1	9.62	92.1		98.5	98.5	89.3	7.66
grzl	95.4	95.4	95.1	80.2	7.8.7	92.4	98.5		100	88.1	98.2
gral	95.4	95.4	95.1	80.2	78.7	92.4	98.5	100	:	88.1	98.2
0221cl	89.6	9.68	89.3	67	76.8	89	89.3	88.1	88.1		89.8
adil.flesh	95.4	95.4	95.1	81.4	79.9	92.4	2.66	98.2	98.2	89.6	

Table 10



BLASTN 2.1.2 [Nov-13-2000]

Reference:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs*, Nucleic Acids Res. 25:3389-3402.

RID: 984591695-10075-13605

Query-

(25 letters)

Database: nt

807,597 sequences: 2,863,827,885 total letters

If you have any problems or questions with the results of this search please refer to the BLAST FAOS

Taxonomy reports

Distribution of 500 Blast Hits on the Ouery Sequence

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Sequences producing significant alignments:	Sca (bi		E Value
gb[AF231651.1 AF231651 Strongylura notata clone HB-82 cytoc	5,0	2e-0	s
gh[AF231650.1]AF231650 Strongylura notata clone HB-159 cyto	_50	2e-0	
ref[NC 002672.1] Dinornis giganteus mitochondrion, complete	_50	2e-0	
ref[NC 00267].1 Emeus crassus mitochondrion, complete genome	<u>5</u> 0	2e-0: 2e-0:	
gb AF232015.1 AF232015 Nothrotheriops shastensis cytochrome gb AF232013.1 AF232013 Bradypus variegatus cytochrome b gen	<u>50</u> 50	2e-0	
	50	2e-0	
gb AY016013.1 Emeus crassus mitochondrion, complete genome gb AY016013.1 Dinornis giganteus mitochondrion, complete g	50	2e-0	5
chlayorsold 11 Dromaius novaehollandiae mitochondrion, part	_50	2e-09	
gb AF230167.1 AF230167 Bonasa umbellus cytochrome b (C/T3)	_ <u>50</u>	2e-09	
gb AF074594.1 AF074594 Baeolophus bicolor cytochrome b gene gb AY005210.1 Poospira melanoleuca isolate 3 cytochrome b	<u>50</u> 50	2e-0	
gb AY005210.1 Poospira melanoleuca isolate 3 cytochrome b gb AY005209.1 Poospira melanoleuca isolate 2 cytochrome b	50	2e-09	
chlavoos208 11 Poospiza melanoleuca isolate 1 cytochrome b	50	2e-09	
gb[AY005205.1] Poospira hispaniolensis cytochrome b (cytb)	<u>50</u>	2e-09	
gb AY005204.1 Poospiza garleppi cytochrome b (cytb) gene,	<u>50</u> 50	2e-09	
gb AY005203.1 Poospiza erythrophrys cytochrome b (cytb) ge gb AY005201.1 Poospiza boliviana cytochrome b (cytb) gene	50	2e-09	
chlavoosigg 11 Poospiza alticola isolate 2 cytochrome b (cy	50	2e-09	
chlavoosigs il Poospiza alticola isolate i cytochrome b (cy	_50	2e-05	
gb AF155870.1 AF155870 Heterocephalus glaber cytochrome b (<u>50</u> 50	2e-05	
gb AF189123.1 AF189123 Glyptotermes eukalypti cytochrome b gb AF102099.1 AF102099 Criniferoides leucogaster cytochrome	<u> 50</u>	2e-05	
gb/AF102099.1/AF102099 Criniferoides leucogaster cytochrome gb/AF102095.1/AF102095S1 Corythaixoides concolor cytochrome	50	2e-05	i
chlaf271065 llAF271065 Mustela erminea specimen-voucher AFL	50	2e-05	
Gb AF243857.1 AF243857 Strongylura notata notata cytochrome	<u> 50</u>	2e-05	
cb AF243856.1 AF243856 Strongylura notata forsythia cytochr	<u>50</u> 50	2e-05	
ref NC 001567.1 Bos taurus mitochondrion, complete genome cb AF306872.1 AF306872 Brachyramphus marmoratus haplotype M	50	2e-05	
cb AF306872.1 AF306872 Brachyramphus marmoratus haplotype M cb AF306871.1 AF306871 Brachyramphus marmoratus haplotype M	50	2e-05	
chia=306870 liaF306870 Brachyramphus brevirostris haplotype	50	2e-05	
gb AF306869.1 AF306869 Brachyramphus brevirostris haplotype	<u>50</u> 50	2e-05 2e-05	
gb AF306868.1 AF306868 Brachyramphus brevirostris haplotype gb AF010406.1 AF010406 Ovis aries complete mitochondrial ge	50	2e-05	
gb AF010406.1 AF010406 Ovis aries complete mitochondrial ge gb AF248662.1 AF248662 Gryllus campestris haplotype 2 cytoc	50	2e-05	
CDIAF248661.1 AF248661 Gryllus campestris haplotype 1 Cytoc	50	Ze-05	
db AF096462.1 AF096462 Rhipidura albicollis cytochrome b ge db AF283644.1 AF283644 Elaphe obsoleta cytochrome b gene, c	<u>50</u>	2e-05	
db/AF283644.1/AF283641 Elaphe obsoleta cytochrome b gene, c	50	2e-05	
chiaf283642.11AF283642 Elaphe obsoleta cytochrome b gene, c	_50	2e-05	
gbla=283641_1/AF283641 Elaphe obsoleta cytochrome b gene, C	<u>50</u> 50	2e-05	
gb AF283640.1 AF283640 Elaphe obsoleta cytochrome b gene, C gb AF283639.1 AF283639 Elaphe obsoleta cytochrome b gene, C	50	2e-05	
gb/AF283639.1/AF283639 Elaphe obsoleta cytochrome b gene, c gb/AF283637.1/AF283637 Elaphe obsoleta LSUMZ 45359 cytochro	50	2e-05	
gb AF283636.1 AF283636 Elaphe obsoleta LSUMZ 44662 cytochro	_50	2e-05	
gb AF283635.1 AF283635 Elaphe obsoleta LSUMZ 40443 cytochro	<u>50</u>	2e-05 2e-05	
gb AF783634.1 AF783634 Elaphe obsoleta LSUMZ 44335 cytochro gb AF783633.1 AF783633 Elaphe obsoleta LSUMZ 42624 cytochro	50	2e-05	
cb AF283632.1 AF283632 Elaphe obsoleta LSUMZ H1911 cytochro	50	Ze-05	
chiaf283631.1 AF283631 Elaphe obsoleta LSUMZ 41197 cycochro	<u>50</u>	2e-05 2e-05	
gb AF28]630.1 AF28]610 Elaphe obsoleta LSUMZ 41189 cytochro gb AF28]629.1 AF28]629 Elaphe obsoleta LSUMZ 41188 cytochro	50	2=-05	
<u>qb AF283629.1 AF283629</u> Elaphe obsoleta LSUMZ 41188 cytochro <u>qb AF283628.1 AF283628</u> Elaphe obsoleta LSUMZ 41187 cytochro	50	2=-05	
gb/AF283627.1/AF283627 Elaphe obsoleta LSUMZ 41186 cytochro	50	2e-05 2e-05	
db AF283625.1 AF283626 Elaphe obsoleta LSUM2 40943 cytochro db AF283625.1 AF283625 Elaphe obsoleta LSUM2 37499 cytochro	<u>50</u>	2=-05	
<pre>gbiaF283625.liaF283625 Elaphe obsoleta LSUME 37499 cytochto gbiaF283624.liaF283624 Elaphe obsoleta LSUME 44480 cytochto</pre>	50	2e-05	
ghiAF283621 liAF283671 Flanhe obsoleta LSUMI 44451 cytochio		2e-05	
gb AF281622.1 AF281622 Elaphe obsoleta LSUM2 40444 cytochro		2e-05 2e-05	
qb AF281621.1 AF281621 Elaphe obsoleta LSUMZ 19925 cytochro qb AF281620.1 AF281620 Elaphe obsoleta LSUMZ 19161 cytochro		20-05	
cb AFZ83619.1 AFZ83619 Elaphe obsoleta LSUME39162 cytochrom	50	20.05	
gb[AF78]618.1[AF78]619 Elephe obsoleta LSUME H15896 cycoche		2e-05	
gbiaf781617 liaf781617 Elaphe obsoleta LSUME H15872 cytoche gbiaf781616 liaf783616 Elaphe obsoleta LSUME 15871 cytocheo		24.05	
gblaf283615 11af283615 Elaphe obsoleta LSUME H15870 cytoche	30	24.05	
gbiaf781614 liaf781614 Elephe obeolete LSUM2 H15867 cycochr		24.05	
gb[AF28]61] 1[AF28]61] Elaphe obsoleta LSUME HISBOR Cytoche	ير	24.02	

Table 9. Animals selected for validation of minimum P'S score for efficient amplification of DNA templates in PCR

SL.	Name	P, S/AFF	P, S/AFR
1	Indian black buck (Antilope cervicapra)	97, 58	96, 54
2	Sheep (Ovis	87, 53	96, 54
3	Pig (Sus scrafa)	87, 52	87, 41
4	Fresh water dolphin (Platanista gangetica)	86, 49	82, 47

	Sc	ore	Ε
Sequences producing significant alignments:	(b:	ics)	Value
gb AF231651_1 AF231651 Strongylura notata clone HB-82 cytoc	50	2e-0	
gb AF231650 1 AF231650 Strongylura notata clone HB-159 cyto	_ <u>50</u> _50	2e-0 2e-0	
ref[NC 002672.1] Dinornis giganceus micochondrion, complete ref[NC 002673.1] Emeus crassus micochondrion, complete genome	_5.0	2e-0	
qb/AF232015 1/AF232015 Nothrotheriops shastensis cytochrome	50	2e-0	S
qb[AF232013.1]AF232013 Bradypus variegatus cytochrome b gen	50	2e-0	
gb[AY016015.1] Emeus crassus mitochondrion, complete genome	50	2e-0	
gb[AY016013.1] Dinornis giganteus mitochondrion, complete g	<u>50</u> 50	2e-0 2e-0	
<pre>gb AY016014.1 Dromaius novaehollandiae mitochondrion, part gb AF230167.1 AF230167 Bonasa umbellus cytochrome b (CYTS)</pre>	<u> 50</u>	2e-0	
qb/AF074594.1/AF074594 Baeolophus bicolor cytochrome b gene	50	2e-0	5 .
gb[AY005210.1] Poospiza melanoleuca isolate 3 cytochrome b	_50	2e-0	
gb AY005209.1 Poospiza melanoleuca isolate 2 cytochrome b	<u> 50</u>	2e-0:	
gb/AY005208.1 Poospiza melanoleuca isolate 1 cytochrome b gb/AY005205.1 Poospiza hispaniolensis cytochrome b (cytb)	<u>50</u> 50	2e-0	
<pre>qb[AY005205.1] Poospira hispaniolensis cytochrome b (cytb) qb[AY005204.1] Poospira garleppi cytochrome b (cytb) gene</pre>	50	2e-0	
gb[AY005203.1] Poospiza erythrophrys cytochrome b (cytb) ge	50	2e-0	
gb AY005201.1 Poospita boliviana cytochrome b (cytb) gene,	_50	2e-09	
gb AY005199.1 Poospiza alticola isolate 2 cytochrome b (cy gb AY005198.1 Poospiza alticola isolate 1 cytochrome b (cy	<u>50</u> 50	2e-09	
<u>qb AY005198.1 </u> Poospiza alticola isolate 1 cytochrome b (cy <u>qb AF155870.1 AF155870</u> Heterocephalus glaber cytochrome b (50	2e-09	
gb AF189123.1 AF189123 Glyptotermes eukalypti cytochrome b	50	2e-05	
gb[AF102099.1]AF102099 Criniferoides leucogaster cytochrome	_50	2e-09	
qb AF102095.1 AF102095S1 Corythaixoides concolor cytochrome	<u>50</u>	2e-09	
gb/AF271065_1/AF271065 Mustela erminea specimen-voucher AF1 cb/AF243857_1/AF243857 Strongylura notata notata cytochrome	50	26-05	_
gb AF243857.1 AF243857 Strongylura notata notata cytochrome gb AF243856.1 AF243856 Strongylura notata forsythia cytochr	50	2e-05	;
refluc 001567.1 Bos taurus mitochondrion, complete genome	50	2e-09	
cb/AF306872.1/AF306872 Brachyramphus marmoratus haplotype M	<u> 50</u>	2e-09	
cb AF306871.1 AF306871 Brachyramphus marmoratus haplotype M cb AF306870.1 AF306870 Brachyramphus brevirostris haplotype	<u>50</u>	2e-05	
cb AF306870.1 AF306870 cb AF306869.1 AF306869 Brachyramphus brevirostris haplotype	50	2e-05	
chlasio6868.11Asio6868 Brachytamphus brevirostris haplotype	50	2e-05	
qb/AF010406.1/AF010406 Ovis aries complete mitochondrial ge	<u>50</u>	2e-05	
gb AF248662.1 AF248662 Gryllus campestris haplotype 2 Cytoc gb AF248661.1 AF248661 Gryllus campestris haplotype 1 Cytoc	50	29-05	
cb AF096462.1 AF096462 Rhipidura albicollis cytochrome b ge	50	2=-05	
gblAF283644.1 AF283644 Elaphe obsoleta cytochrome b gene, C	_50	2e-05	
cb AF283643.1 AF283642 Elaphe obsoleta cytochrome b gene, c gb AF283642.1 AF283642 Elaphe obsoleta cytochrome b gene, c	_ <u>50</u> 50	2e-05	
<pre>gb AF283642.1 AF283642 Elaphe obsoleta Cytochrome b gene, C gb AF283641.1 AF283641 Elaphe obsoleta Cytochrome b gene, C</pre>	50	2e-05	
gblAF283640.1 AF283640 Elaphe obsoleta cytochrome b gene. C	50	2=-05	
gb/AF283639.1/AF283639 Elaphe obsoleta cytochrome b gene, c	<u> 50</u>	2e-05 2e-05	
gb AF283637.1 AF283637 Elaphe obsoleta LSUMZ 45359 cytochro gb AF283636.1 AF283636 Elaphe obsoleta LSUMZ 44662 cytochro	<u>50</u> 50	20-05	
gblAF283635.1 AF283635 Elaphe obsoleta LSUMZ 40443 cytochro	50	2e-05	
gb/AF283634.1 AF283634 Elaphe obsoleta LSUMZ 44335 cytochro	_50	2e-05	
gb[AF283633.1[AF28363] Elaphe obsoleta LSUMZ 42624 cytochro gb[AF283632.1[AF283632 Elaphe obsoleta LSUMZ K1911 cytochro	<u>50</u>	2e-05	
gb AF783632.1 AF283632 Elaphe obsoleta LSUM2 K1911 cytochro gb AF783631.1 AF283631 Elaphe obsoleta LSUMZ 41197 cytochro	50	2e-05	
gblaF283630.1 AF283630 Elaphe obsoleta LSUME 41189 cytochio	50	2=-05	
gb/AF283629.1/AF283629 Elaphe obsoleta LSUME 41188 cytochro	<u>50</u>	2e-05 2e-05	
gb AF283628.1 AF283628 Elaphe obsoleta LSUMZ 41187 cytochro gb AF283627.1 AF283627 Elaphe obsoleta LSUMZ 41186 cytochro	50	2=-05	
gb/AF281626.1/AF281626 Elaphe obsoleta LSUMZ 40941 cycochro	50	2e-05	
gb/AF283625.1/AF281625 Elaphe obsoleta LSUMZ 37499 cytochro	50	2e-05 2e-05	
gb AF28]624.1 AF28]624 Elaphe obsoleta LSUMZ 44480 cytochro gb AF28]62].1 AF28]62] Elaphe obsoleta LSUMZ 44451 cytochro	<u>50</u>	2-05	
gb AF28]622.1 AF28]622 Elaphe obsoleta LSUM2 40444 cytochro	50	Ze-05	
gb[AF28]621.1[AF28]621 Elaphe obsoleta LSUME 19925 cytochro	50	2÷-05 2e-05	
qb[AF2q]620, 1[AF28]620 Elaphe obsoleta LSUM2 37163 cytochro qb[AF2q]619, 1[AF28]619 Elaphe obsoleta LSUM237162 cytochrom	<u>50</u> 50	Ze-05	
gb AF283618.1 AF283618 Elaphe obsoleta LSUME H15876 cytochr	20	20.05	
gblaf781617.1[AF281617 Elaphe obsoleta LSUME Hisere cytochr	50	Ze-05	
gbiaf783616 liaf781615 Elaphe obsoleta LSUM2 15871 cytochro gbiaf783615 liaf783615 Elaphe obsoleta LSUM2 H15870 cytochr	<u> 52</u>	Ze-05 Ze-05	
gblaf2g]614 llaf28]614 Elaphe obeoleta LSUME H15687 cytochr	50	:05	
ablafield 1 AFIELD Elaphe obsoleta LSUM2 Hisee cycoche	_52	34.05	

	and a subfigure out come	so	2e-05
gb[AF183612.1]AF283612	Elaphe obsoleta LSUMZ H15884 cytochr	50	2e-05
qh[AF28]611.1[AF28]611	Elaphe obsoleta LSUM2 H15031 cytochr	50	2e-05
gb AF283610.1 AF283610	Elaphe obsoleta LSUMZ H15010 cytochr	50	2e-05
gb AF283609.1 AF283609	Elaphe obsoleta CAS 169468 cycochrom Elaphe obsoleta LSUME H14782 cycochr	50	2e-05
gb[AF283608.1[AF28]608	Elaphe obsoleta LSUME H14781 cytochr	50	2e-05
95 AF281607.1 AF283607	Elaphe obsoleta LSUME H14724 cycochr	50	2e-05
gb AF283606.1 AF283606	Elaphe obsoleta cytochrome b gene. C	50	2e-05
gb[AF283605.1[AF283605	Elaphe obsoleta cytochrome b gene. C	50	2e-05
gb AF283604 . 1 AF283604	Elaphe obsoleta cytochrome b gene, C	50	2e-05
95 AF283603.1 AF283603	Elaphe obsoleta LSUME H3388 cytochro	50	2e-05
95 AF283602.1 AF283602	Elaphe obsoleta LSUME H3385 cytochro	50	2e-05
95 AF283601.1 AF283601	Elaphe obscieta LSUMZ H3384 cytochro	_50	2e-05
gb AF283600.1 AF283600	planks hairdi LSUME H3382 Cytochrome	_50	2e-05
gb AF283599 . 1 AF283599	Flacks hairdi LSUMZ H3381 cytochrome	_50	2e-05
95 AF283598 1 AF283598	clarke checleta LSUMZ H3379 Cytochio	_50	2e-05
gb AF283597.1 AF283597 gb AF283596.1 AF283596	Flanks shediera LSUMZ 39616 cycochio	50	2e-05
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gb AF283594.1 AF283594	Flanke obsoleta LSUMZ H3345 CYCOCHIO	<u> 50</u>	2e-05 2e-05
gb AF283593.1 AF283593	Flanka sheelera LSUMZ H3309 Cytochio	50	2e-05
GD AF283592.1 AF283592	Flanks checlera LSUMZ H3306 Cytochio	<u>50</u>	2e-05
Sb AF283591.1 AF283591	Flamba obsoleta LSUMZ H3Z76 CYCOCREG	<u>50</u>	2e-05
gb AF283590.1 AF283590	Elaphe obsoleta LSUMZ H3246 cytochro	50	2e-05
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cb AF283587.1 AF283587	Elaphe obsoleta LSUMZ H3206 cytochro	50	2e-05
CD AF283586.1 AF283586	Elaphe chsoleta LSUMZ H3191 cytochro	50	2e-05
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GD AF283584.1 AF283584	Elaphe obsoleta LSUMZ H3188 cytochro	50	2e-05
GD AF283583.1 AF283583	Elaphe obsoleta LSUME H3186 cytochro	50	2e-05
GD AF283582.1 AF283582	Elaphe obsoleta LSUMZ H3169 cytochro	50	2e-05
cb AF283581.1 AF283581	Flanks obsoleta CAS 203083 CytoChrom	<u>50</u>	2e-05
GD AF282580.1 AF283580 GD AF283579.1 AF283579	Flanka obsoleta CAS 203079 Cytochiom	_50	2e-05
gb AF283578.1 AF283578	Flanka checiera LSUMZ H2286 Cytochio	<u> 50</u>	2e-05
GD AF283570 . 1 AF283577	Flanka checieta CAS 208631 cytochrom	_50	2e-05 2e-05
S5 AF283576.1 AF283576	Planta checlera LSUMZ H2229 Cytochio	<u> 50</u>	2e-05
GD AF187030.1 AF187030	phinophylla numilio isolate TK46001	<u>50</u> 50	2e-05
cb[AF310052.1[AF310052	Poospiza hispaneolensis cytochrome b	50	2e-05
CD AF310046.1 AF310046	Volatinia jacarina cytochrome b gene	50	2e-05
GD AF171919.1 AF171919	Deinagkistrodon acutus cytochrome b Trimeresurus mucrosquamatus cyth gen	50	2e-05
G51AF171897.1 AF171897	Agelaius cyanopus cytochrome b (cytb	50	2e-05
gb[AF290174.1[AF290174	Agelains phospiceus cytochrome D (Cy	50.	2e-05
gb AF290173.1 AF290173	onicestus major evtochrome b (cyta)	50	2e-05
GD AF290171.1 AF290171 GD AF290170.1 AF290170	amblyce-cus holosericeus cytocarome	50	2e-05
gb AF290150.1 AF290150	Volarinia jacazina cytochrome D (CYE	50	2e-05 2e-05
95 AF176252.1 AF176252	peich-odoptomys zacatecae cytochiome	<u>50</u>	2e-05
gb[AF176251.1[AF176251	pairbrodogromve zacatecae cytochiome	<u>50</u> 50	2e-05
gb AF163907 . 1 AF163907	Microtus manthognathus cytochrome b	<u>50</u>	2e-05
CD AF163904.1 AF163904	Microtus pinetorum cytochrome b gene Microtus ochrogaster cytochrome b ge	50	2e-05
gb AF163901.1 AF163901	Microtus miurus cytochrome b gene. c	50	2e-05
95 AF153899.1 AF163899	Microtus californicus cytochrome 8 (50	2e-05
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95 AF288524 . 1 AF288524	ningabelus dussumieri isolate Germa	5.7	Ze-05
GD AF288523.1 AF288521	ninconstructura desgramieri isolate waite	50	2e-05 2e-05
95 AF288522.1 AF288522	nimembalve dusqumieri isolate ALGY	<u>50</u>	2e-05
GD AF123530 . 1 AF123530	poilogogo pyrolophus cytochrome D (50	2e-05
95 AF123512.1 AF133512	Eubucco bourcierii Eucinkae cytochro Adolfus vauereselli cytochrome b gen	50	2e-05
95 AF206548 . 1 AF206548	Cymnorhina Cibicen cycochrome b gane	5.7	2e-05
95 AF197867.1 AF197867	ties autopaea cytochrome b gene, part	<u>50</u>	20-05
Tion	to complete micochondrion, complete	50	2e-05
Cating ODIGEL II Dasy	wa sovemeinceus mitochondrion, compie	<u> 50</u>	2e-05 2e-05
GDIAF141217. LIAF141217	negretal income to country Tanzania Cy	<u>50</u>	2e-05
GDIAF201615.1 AF201615	Pancodon buchholzi cyoschrome b gene	<u>50</u>	20-05
gb AF077920 1 AF077920	Bombus nevadensis cycochrome b gene Oreamnos americanus cycochrome b (cy	20	2=-05
qb[AF1906]2.1[AF1906]2	taurus mitochondrion, complete genome	50	24-05
951J01194 1180VHT 800			
951AF191810.11AF191810	Cochlearius cochlearius cytochrome b	22	20-05

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50 Ze-05
   gb/U89181.1 [CAU8918] Chlorostilbon aureoventris cytochrome ...
                                                                                     _$0 2e-05
   gb|U84171.1|AFU89171 Asio flammeus cytochrome b (cytb) gene...
                                                                                    _50 2e-05
   gb[AF217633.1]AF217833 Homoroselaps lacteus cytochrome b ge...
                                                                                      50 2e-05
   gb|AF217822.1|AF217822 Hydrophis semperi cytochrome b gene,...
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  qb[AF217813.1]AF217813 Acanthophis antarcticus cytochrome b...
                                                                                    50 2e-05
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  gb[AF220408.1]AF220408 Calliophis kelloggi cytochrome b (cy...
  qb[AF126430.1[AF126430 Ellobius fuscocapillus cytochrome b ...
                                                                                    50 2e-05
50 2e-05
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  gb[AF059111.1|AF059111 Sarkidiornis melanotos cytochrome b ...
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  gb[AF059053.1[AF059053 Aix sponsa cytochrome b gene, partia...
                                                                                     _50 2e-05
  qb[AF099308.1]AF099308 | Icterus wagleri wagleri cytochrome b...
  gb|AF099295.1|AF099295 Icterus gularis yucacanensis cytochr...
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  gb|AF099294.1|AF099294 | Icterus gularis tamaulipensis cytoch...
                                                                                    _50 2e-05
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50 2e-05
  gb|AF160610.1|AF160610 Cricetomys emini Cemi636 cytochrome ...
  gb|AF036280.1|AF036280 Tragelaphus strepsiceros cytochrome ...
gb|AF036277.1|AF036277 Tragelaphus scriptus cytochrome b (c...
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  gb|AF194218.1|AF194218 Phrynosoma platyrhinos cytochrome b ...
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  gb[AF194216.1[AF194216 Urosaurus ornatus cytochrome b gene,...
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  ref NC 001941.11 Ovis aries mitochondrion, complete genome 50 2e-05
ref NC 000877.11 Aythya americana mitochondrion, complete g... 50 2e-05
ref NC 000846.11 Rhea americana mitochondrion, complete genome 50 2e-05
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  gb[AF089058.1]AF089058 Quiscalus quiscula cytochrome b (cyt...
                                                                                          2e-05
                                                                                    50
                                                                                          2e-05
  gblaF089055.1|AF089055 Quiscalus major cytochrome b (cytb) ...
                                                                                     50 2e-05
  gb|AF089054.1|AF089054 Quiscalus lugubris cytochrome b (cyt...
gb|AF089046.1|AF089046 Oreopsar bolivianus cytochrome b (cy...
                               Quiscalus lugubris cytochrome b (cyt...
                                                                                   50 2e-05
 cb|AF089042.1|AF089042 Molothrus badius cytochrome b (cytb)... 50 2e-05
                               Macroagelaius imthurni cytochrome b ... 50 2e-05
Lampropsar tanagrinus cytochrome b (... 50 2e-05
 CD AF089039.1 AF089039
 G5 | AF089037.1 | AF089037
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50 2e-05
 cb|AF089026.1|AF089025
gb|AF089025.1|AF089025
                                Gymnomystax mexicanus cytochrome b (...
                               Gaorimopsar chopi cytochrome b (cytb...
                                                                                  50 2e-05
 c5|AF089024.1|AF089024
                               Euphagus cyanocephalus cytochrome b ...
                                                                                   50 2e-05
50 2e-05
                               Euphagus carolinus cytochrome b (cyt...
 cb|AF089023.1|AF089023
 cb|AF089021.1|AF089021
                               Dives warszwewiczi cytochrome b (cyt...
                                                                                   50 2e-05
 G5|AF089020.1|AF089020
                               Curaeus curaeus cytochrome b (cytb) ...
                                                                                   50 2e-05
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50 2e-05
 cb|AF089016.1|AF089016 Amblycercus holosericeus cytochrome ...
 cb/AF089013.1/AF089013 Agelaius xanthophthalmus cytochrome ...
 cb[AF089012.1|AF089012 Agelaius xanthomus cytochrome b (cyt...
                                                                                   <u>50</u> Że-05
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                                                                                   50 2e-05
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 cb|AF089005.1|AF089005 Agelaius cyanopus cytochrome b (cytb...
 GD/AF108695.1/AF108696 Scolomys juruaense cytochrome B (cyt...
GD/AF108685.1/AF108685 Wiedomys pyrrhorhinos cytochrome B (...
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 cb[AF108677.1]AF108677 Thomasomys oreas cytochrome B (cyt3)...
                                                                                   50 2e-05
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 gb|U89627.1|9MU89627 Bolitoglossa marmorea cytochrome b (cy...
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gb[U9010].l[OMU9010] Ovibos moschatus cytochrome b (cytb) g...
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cb[U90302.1]OMU90302 Ovibos moschatus cytochrome b (cytb) g...
gb[U90301.1[OMU90301] Ovibos moschatus cytochrome b (cytb)| g...
gb[U90300.1[OMU90300] Ovibos moschatus cytochrome b (cytb)| g...
                                                                                   50 Ze-05
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gb[AF038883.1[AF03888] Deinagkistrodon acutus cytochrome b ...
gb[AF0]9269.1[AF0]9268 Agkistrodon concortrix cytochrome b ...
gb[AF0]9267.1[AF0]9267 Boa constrictor cytochrome b (cytb) ...
                                                                                    50 2e-05
                                                                                   50 2=-05
gb[S49215.1]S49215 apocytochrome b (sheep, domestic, Merino...
                                                                                   50 2e-05

50 2e-05

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gb|AF158698.1|AF158698
Geomys pinetis cytochrome b gene. co...
gb|AF158692.1|AF158692
Geomys bursarius jugossicularis cyto...
gb|AF058193.1|AF068193
Ichaginis cruentus cytochrome b loyt...
gb/AF091629.1/AF091629 Ancilocapra americana cytochrome b (...
gb[AF02205] 11 Tragelaphus strepsiceros Cytochrome b (cytb) ...
gb[AF022052 11 Tragelaphus derbianus cytochrome b (cytb) ge ...
gb[AF022050 11 Hippotragus equinus cytochrome b (cytb) gene ...
gb[AF022057 11 Tragelaphus oryx cytochrome b (cytb) gene ...
gbiaFilisco liaFilisco Lagenorhynchus acutus isolate LACU74 ...
gbiaFilisco liaFilisco Lagenorhynchus acutus isolate LACU73 ...
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50 2e-05
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gb NESE 16. 1 ENGS 16
gb NCS 408. 1 ENGS 16
gb NCS 408. 1 ENGS 16
gb NCS 408. 1 ENGS 19

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                                                                                                     _SO 2e-05
                                                                                                      50 2e-05
                                                                                                     50 2e-05
                                                                                                      50 2e-05
                                                                                                     _50 2e-05
gb|U69794.1|ESU69794 Epicrates striatus mecraniei cytochrom...
gb|U69791 1|ESU69792 Epicrates striatus mccraniei cytochrom...
gb|U69791 1|EMU69792 Epicrates monensis cytochrome b (cytb)...
                                                                                                     <u>50</u> 2e-05
                                                                                                     _<u>50</u> 2e-05
gb|U69790.1|EMU69790 Epicrates monensis cytochrome b (cytb)...
                                                                                                     50 2e-05
gb|U69786.1|EFU69786 Epicrates ford: cytochrome b (cyto) ge...
                                                                                                     <u>50</u> 2e-05
                                  Epicrates fordi cytochrome b (cytb) ge...
                                                                                                     50 2e-05
50 2e-05
50 2e-05
gb|U69784_1|EFU69784 Epicrates fordi cytochrome b (cytb) ge...
gb|U69779_1|ECU69779 Epicrates cenchria cytochrome b (cytb)...
gb]U69777.1 ECU69777 Epicrates cenchria cytochrome b (cycb)...
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gb | U69776 . 1 | EAU69776
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gb | U69774 . 1 | EAU69774
                                  Corallus envirs cytochrome b (cytb) g...
                                                                                                     _50 2e-05
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                                  Corallus envirs cytochrome b (cytb) g...
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95 U69771 . 1 CEU69771
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gb | U69770 . 1 | CEU69770
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gb|U69769.1|CEU69769 Corallus envdris cytochrome b (cytb) g...
gb1U69752.1|CAU69752 Candoia aspera cycochrome b (cycb) gen...
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gb]U69746.1|3CU69746 Boa constrictor cytochrome b (cytb) ge...
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gb|AF090339.1|AF090339 Rhea americana mitochondrion, comple...
cblaF006275.1/AF006275 Chemidophorus tigris strain Isla Ang...
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cb[AF006267.1[AF006267 Cnemidophorus tigris strain Isla Smi...
                                                                                                     <u>50</u> 2e-05
cb|AF034969,1|AF034969 Connochaetes taurinus cytochrome b g...
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50 2e-05
gb|AF029822.1|AF029822 Alcelaphus buselaphus cytochrome b g...
gblaF028821.1|AF028821 Damaliscus lumatus cytochrome b gene...
SD AF061340.1 AF061340 Artibeus jamaicensis mitochondrial D...
                                                                                                     <u>50</u> 2e-05
gb|AF076093.1|AF076093 Thalassarche impavida cytochrome b (...
                                                                                                     _50 2e-05
                                                                                                     50 2e-05
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                                     Thalassarche carteri cytochrome b (c...
G5 | AFG76091 . 1 | AFG76091
gb|AF076072.1|AF076072 Pelagodroma marina cytochrome b (cyt...
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50 2e-05
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GD | AF076063 . 1 | AF076063
gb|AF076059.1|AF076059 Hydrobates pelagicus cytochrome b (C...
SD|AF076056.1|AF076056 Garrodia mereis cytochrome b (cytb) ...
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gb|AF076053.1|AF076053 Fregeria tropica cytochrome b (cytb)...
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gbla-076050.1|a-076050 Diomedea gibsoni cytochrome b (cytb) ...
gblar076049.11AF076049 Diomedea epomophora cytochrome b (cy...
                                                                                                     <u>50</u> 2e-05
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                                                                                                     50 2e-05
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50 2e-05
                                     Diomedea antipodensis cytochrome b (...
G51AF076047:1|AF076047
gb|UE3314.1|MSU83314 Micrastur semitorquatus cytochrome b (...
                                                                                                      <u>50</u> 2e-05
gb/UB3318.1|MEU83318 Microhierax erychrogenys cycochrome b ...
                                                                                                     50 2e-05
gb1U37303.1|SAU37303 Synthliboramphus antiquus cytochrome b...
                                                                                                     50 2e-05
50 2e-05
                                  Ptychoramphus aleuticus cytochrome b g...
951037302.11PAU37302
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G5 | U37296 .1 | C2U37296
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55 U37289:1 | BBU37289
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                                  Aethia pusilla cytochrome b gene. mito...
95 U37104 . 1 A2U37104
95 U37087 . 1 ACU37087
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951487525.1 HGU87525
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G51U87524.1|HGU87524
                                 Heterocephalus glaber cytochrome-b gen...
G5 | U87523 . 1 | HGU87523
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g5| U87522.1 | HGU87522
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G5 [U] 7964.1 | STU17864
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50 2e-05

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G5 | U17861.1 | QAU17863
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95 U55274 . 1 TBU65274
                                 Thomomys boccas cytochrome b (cytb) gs...
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                                  Thomomys bottae cycochrome b (cyth) ge...
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                                                                                                     50 2e-05
50 2e-05
gb[AF0]47]9.1[AF0]47]9 Capra aegagrus cytochrome b (cyth) g...
gb[AF0]47)9.11 Capra caucasica cytochrome b (cytb) gene, mi...
gb[AF0]47)7.11 Capra cytindricornie cytochrome b (cytb) gen...
                                                                                                     qb[AF0]47]4 1[AF0]47]5 Capra falconer: cytochrome b (cytb)
TDIAFF114715 11 Capra Lbex cytochrome b (cytb) gene. mitocho...
_5<u>.</u>2
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gb[AF03470F.1] Ovis dall: dall: cytochrome b (cyth) gene. m	50	2e-05
ghiarcation of our daily daily cycochrome b (cyco) gene, ghiarcation of our ammon darwing cycochrome b (cyco) gene,	_ <u>5</u> 0	26-05
ght AF034717 11 Ovis ammon darwini cytochrome b (cyto) gene	_50	2e-05
gb[AF034714.1]AF034714 Pantholops hodgsoni cytochrome b (cyto) gb[AF057131.1]AF057132 Taxides taxus cytochrome b (cytb) ge	.50	2e-05
	_50	2e-05
gt. 1144805 1 TMU94805 Trogon melanurus cytochrome b gene, mi	50	20-05
This can a litruganda Trogon Complus Cycochiome D gene, material	50	2e-05
Lineage 1 Thirdael Troppe Ville's Cytocarome o Sene, mitter	_	2e-05
Thirmness 1 (amones) Sericossypha albocristata cytochrome	<u>50</u>	2e-05
The state of the s	<u>_50</u>	
The control of the co	5_0	26-05
	_50	20-05
	_50	2e-05
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	50	2e-05
	50	2e-05
	50	2é-05
ch AF006212.1 AF006712 Buthraupis montana Cytochiome D (Gyt)	50	2e-05
emblaJ293419.1[RRU293419 Rupicapra rupicapra rupicapra rupicapra	50	2e-05
The sandy and a foot-did to Killicabia by tempted management	50	2e-05
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	_50	2e-05
the radacta tipermanata Rupicapra rupicapra rupicapra mito	_50	2e-05
to record vices 202418 Capra falconeri mitochondrial part	_50	26-03
The state of the s	_50	2e-05
the top the the state of the st	_50	2e-05
Liviscos ileguiseas schilbe mystus mitochondriai cyth gen	_50	2e-05
	_50	2e-05
	<u>_50</u>	2e-05
	_50	2e-05
	50	Ze-05
	50	2e-05
cb/U76052.1 DNU75052 Dromaius novaehollandiae cytochrome b	50	2e-05
emb[AJ236634.1]CGL236834 Clethrichomys glareolus mitochondr	50	2e-05
chlu83158.1 POU83158 Pelecanus onocrotalus cytochrome B gen	50	2e-05
chiusiss.iipousiss Pelecanus onocrotalus cytochrome B gen	50	2e-05
cblu83156.1 AAU83156 Anhinga anhinga cytochrome B gene, mit	50	2e-05
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cb[U81356.1]CLU81356 Chelodina longicallis cycochiame b game	50	2e-05
emblaJ277676,1 ESC277676 Elaphe scalaris micochondrial parc	50	Ze-05
emblaJ277675.11ESC277575 Elaphe scalaris mitochondrial part	5 C	29-05
emb[AJZ77672.1]ELO277672 Elaphe longissima mitochondrial pa	50	29-05
emb[AJ277671.1]ELO277671 Elaphe longissima mitochondital pa	50	2=-05
emb[Y11832.1[MTDNCOMON Dasypus novemcinetus complete mitoch	50	2=-05
emb 11632:1 10823601 Nemacheilus barbaculus mischondria	50	20-05
emb[AJ388468.1[IME389468 ICTAIUTUS METAS MITTERIORETTA	50	2e-05
Listance directories leuraspius delimeatus mittalundante.	50	2e-05
gb] U46167, 1] SCU46167 Sciurus carolinensis cytochrome b gene	50	2e-05
emb[AJ24567] 11SIN245671 Schilbe incermedius partial mitoch	50	2e-05
emb AJZ45538.1 SIN245638 Schilbe incermedius partial mitoch	50	2=-05
	50	2=-05
emb[AJZ45677,1]EDE245677 Eutropius depressirostris partial	50	2e-05
emb[AJ345576.1[5D6245676 Eutropius depressirostris partial	50	2e-05
emblAJ245575 1 EDE245675 Eutropius depressirostris partial emblAJ245674 1 EDE245674 Eutropius depressirostris partial	30	2e-05
	50	2e-05
the second of th	50	Ze-05
	50	2e-05
	50	2=-05
	50	20-05
	50	2e-05
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The same of the sa	_50	2=-05
	_50	20-05
This age tipaliages Comedes emsterdamensis cytochrome b 1	50	20.05
liverse il politerse piranga mabra cycochesme b gene, misse	-20	205
luceing illamicesna Artibeus planitostria cytochrome b (27	-52	24-05
entities of tlantices of A-sibeue obscurue cytochrome b (tyto)	-20	: - · 05
gb1955305 11AQU55595 Artibeus obscurse cytochrome b (cyto)	777	3e - 95

gb[UECSUS.1]ALUGGSOS Artibeus lituracus cytochrome b (cytb)	50	2e-05
	50	2e-05
	50	2e-05
	50	2e-05
gb[U66502.1]AIU66502 Arcibeus incermedius cycochrome b (cyc	50	2e-05
db[U66501.1]AIU66501 Arcibeus inopinatus cytochrome b (cytb		2e-05
Thirteson tituliseson Arribeus hirsutus Cytochiome C (Cyto)	50	2e-05
Thinkered I Laminered Attiheus fracerculus cytochrome o leye	50	
chiliseage liamiseage Artibeus timbriatus cycochrome b (cytb	. 50	2e-05
gb[U61061.1[93U6306] Brachyramphus breviroscris cycochrome	<u> 50</u>	2e-05
	50	2e-05
	50	20-05
	50	2e-05
	50	2e-05
gb U58386_1 SJU58386 Scolomys juruaense cycochrome b (cyc-b	50	2e-05
db L11905.1 CGYMTCYTBD Cratogeomys gymnurus mitochdnoria: C		2e-05
Thirder I IMMIRAGT? Merachirus nucicaucatus cytochiome o 1	. <u>50</u>	2e-05
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THE TAGES SIMPLIAGES CARRELUS CARRELIUS MITOCRORETIAL CYL	_50	2e-05
The large of the control of the caprelles caprelles mitochondrial cyt	_50	2=-05
- Liviana il Covercy Craregeomys tylorhinus mitochondrial	<u>. 5</u> 0	2e-05
GD/L11901 1/ PPGMTCYTBB Geomys bursarius juggosicularis mito	_50	2=-05
	_50	2e-05
	50	2e-05
gb U46183.1 SSU46181 Sciurus stramineus cytochrome b gene,	50	2e-05
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emb X95767.1 NGRIMC9 N.griseus mitochondrial cytochrome b gene	_ <u>50</u>	Ze-05
	_50	2e-05
embly86754 MTLCCVT17 L. Crumeniferus mitochondriai Cyto gene	_50	2e-05
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ductagorsada 1/22015242 Pantodon buchholzi mitochondrial Cy	_50	2e-05
The lycone il Mitters I dorbigny mitochondrial gene for C	_50	2e-05
TOROGRA IMPETOVERS PARCIFET LARANGUS mitochondrial Cy	_50	2e-05
	_50	2e-05
	50	2e-05
emb V00654.1 MIBTXX Bos raurus complete mitochondrial genome	50	2e-05
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95/U27543.1/BRU27543 Balearica regulorum cytochrome b (cytb	50	2e-05
dbilA9030025.1/A8030025 Sciurus stramineus mitochondrial cy	50	2e-05
cblu18258.115CU18258 Spharagemon campestris cycochrome b ge	50	2=-05
qb/u18257.1/scul8257 Spharagemon collare cytochrome b gene	50	2=-05
gb U1825].1 TPU1825] Trimerocropis pistrinaria cytochrome b	50	205
Gb[U18250.1 CPU18250 Cammula pellucida cytochrome b gene, m	50	2e-05
qb/U17904.1/CCU17904 Circotectix carlinianus mitochondrion	<u> 50</u>	20-05
dbj D84202.1 GOTMTCBB Capra falconeri mitochondrial DNA for	50	2=-05
Child82889.1082889 Bos javanicus micochondrial DNA for cyt	50	2=-05
dbil032195.1/CCRMTCB25 Capricornis sumatrensis mitochodrial	50	2e-05
Colloscial ilCCRMTCB21 Capricomis crispus mitochondrial ge	50	2=-05
Cervus elaphus kansuensis micochond	50	2=-05
db1 AB021097.1 AB021097 Cerrus elaphus xanthopygus mitochon	50	2e-05
dbi[AB021095.1]AB021095 Cervus nippon yesoensis mitochondri	50	2=-05
db AB021094_1 AB021094 Cervus mippon centralis mitochondri	50	24-05
db1[AB021092.1[AB021092 Cervus nippon mageshimae mitochonds	50	2e-05
dbj[AB021091.1]AB021091 Cervus mippon keramae mitochondrial	50	20-05
Col ABOO1612.1 ABOO1612 Cervus elaphus mitochondrial DNA to	50	205
dbil094205.115HPMTCBE Sheep misochondrial DNA for cycochrom dbil094203.115HPMTCBC Ovie musimon misochondrial DNA for cyc.	20	205
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dry Diller.1 CEDMTCELL Cervus nippon milochondrial gene for	50	26-05
dri Dille: CEUMTCEIL Cervus nippon mitochondrial gene lot dhi ABOI 1986 ABOI 1984 Cervus elaphus scottcus mitochondri	_50	26-05
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dbj AB021096 1 AB021096 Cervus elaphus Canadensis mitochond	50	2e-05
ALLIEBRATORS ILERATIONS CARRIER RIDEOR RIDEOR MICOCHORCIAL		2e-05
and reason to the process of the party of th	_50	
dbj AB008535 1 AB008535 Dinocon semicarinatus micochandrial	_5,0	2e-05
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dbj AB006800.1 AB006800 Ovis aries mitochondrial DNA 132 Cy	_50	2e-05
gb L12763.1 LDHMTCYTB Lepidochelys kemp1 (LK-3) micochondri	50	2=-05
This access the provide the carehathinus blumbeus misbenones and the commentation of the comments of the comme	-	2e-05
autracoat timpove Handerma bilabatum cycochrome b gene	_5 C	2e-05
This again a lamera Chiroderma doctae evicentome o gene, a com-	_50	
The ranges alcayologs Casta avrenaica (individual 12) Mi	<u>_50</u>	2e-05
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emb AJ010051_1 CPY010051 Capra pyrenaica (individual 5) mit	50	2e-05
The totage of the votage of the control of the cont		2e-05
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TOTOLOGIA 1 COVOLOGER Carra evrenaica (individual 5) mic	<u>_5</u> 3	2e-05
the response story of the story	_50	2e-05
	50	2e-05
gb U08946.1 CAU08946 Coragyps atratus mitochondrion cytochr	50	2e-05
Lucane timerous Cacharres hurrovianus mitochoncilon Cy		2e-05
The same of the sa	<u> 50</u>	2e-05
planales alba micochondrion cytochiome	_5C	2e-05
Transplant Property Phoenicapterus ruber mitochondrion Cyt	_50	
leaching illames a cristatus mitochondrial cytochrome b gene	<u> 50</u>	2e-05
thecare through a bennettii mitochondrial cytochrome b gene	<u>50</u>	2e-05
	<u> 50</u>	2e-05
	<u> 43</u>	8e-05
	45	3e-04
cb AF157466.1 AF157466 Lepus timidus cytochrome b (Cyb) gen	45	3e-04
cb AF157465,1 AF157465 Lepus granatensis cytochrome b (C/b)	45	3e-04
cb AF157464 1 AF157464 Lepus corsicanus haplocype 1 cytochr	45	3e-04
The server is result the considering haplotype is the constant	46	3e-04
The series of the series of the series and acts of the series of the ser	46	3e-04
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Tylogymis crocodilus clone H3-155 CY	45	
Transcenting Transcent Transcenting acus pacificus cytochrome	_======================================	3e-04
Transce themselves Transce Melanotus clone S	43	34-04
Tylosurus acus melanotus clone S.A	<u> </u>	3e-04
	45	3e-04
	45	3e-04
	45	3e-04
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	<u> 45</u>	3e-04
	45	Je-04
	45	3e-04
	-55	3e-04
	45	30-04
	45	304
	45	Je-04
	45	304
	45	3=-04
The second secon	45	Je-04
	45	304
The state of the s	* 5	30-04
95 AF318555.1 AF318556 Alligator mississippiensis isolate S	45	34-04
gb/AFJ18555 .1/AFJ18555 Alligator mississippiensis isolate 5	45	3=-04
gb[AF]18554.1[AF]18554 Alligator mississippiensis isolate 5	45	Je-04
gtlAF)1855).1[AF)1855] Alligator mississippiensio isolate C	4.5	304
gblaF118552 .1laF118552 Alligator mississippieness inclair to	45	304
gblaffi8551.llaff18551 Alligacor miserssippiensis isolate L	45	34-04
dbiAF318550.11AF319550 Alligator misologippienolo logiste F	45	304
	4.5	304
Name and the second sec	<u></u>	14.04
qt[AFT]904: [[AFT]804] Sorex monticulus specimen. Vouchet Art.		

gb AF326271.1 AF326271 gb AF326270.1 AF326270 gb AF326266.1 AF326266	Myospalax myospalax tytochr Myospalax psilurus isolate 2 cytochr Myospalax psilurus isolate 1 cytochr Eospalax fontanierii isolate 4 cytoc Acrocephalus dumetorum mitochondria	46 46 46 44	3e-04 3e-04 3e-04 0.001
emb[a.T004340.1]ADAJ4264	Acrocephalus dumetorum mitochondria	-44	0.001

Alignments

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AF230167	266		290 230
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AY005209	290		314
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AY005204	290		314
AY005203	290		314
AY005201	290 290		314
AY005199	290		314
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AF102095	208		232
AF271065	398	**********	422
AF243857	275		299
AF243856	275		299 14935
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AF306872	302		326
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AF283615	374		398
AF283634	374		398
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AF283632	374		378 398
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AF283630	374 374		328
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AF283627	374		338
AF283625	374		398
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AF283524	374		398 398
AF202622	374		378
AF201622	374 374		376
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AF281618	374	•••••	. 398
AF283617	374	• • • • • • • • • • • • • • • • • • • •	. 398
AF283616	374		
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AF283614	374	• • • • • • • • • • • • • • • • • • • •	
AF283613	374		
AF283612	374		. 398
AF283611	374		
AF283610	374		
AF283609	374		
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AF283606	374		
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AF283604	374 374		
AF283603	374		398
AF283602 AF283601	374		
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AF283599	374		398
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AF283597	374		398
AF283596	374		398
AF283595	374	• • • • • • • • • • • • • • • • • • • •	398
AF283594	374	• • • • • • • • • • • • • • • • • • • •	398
<u>AF283593</u>	374		398
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AF283591	374	•••••	398
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AF283589	374		398
<u>AF283588</u> AF283587	374		398
AF283586	374		398
AF283585	374		398
AF283584	374	• • • • • • • • • • • • • • • • • • • •	398
AF283583	374		398.
AF283582	374.	••••••	398
<u>AF283581</u>	374	• • • • • • • • • • • • • • • • • • • •	398
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AF283578 AF283577	374 374		398
AF283576	374		398
AF187030	398		422
AF310052	299		323
AF310046	299		323
AF171919	302	• • • • • • • • • • • • • • • • • • • •	326
AF171897	297	•••••	321 305
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AF290171	261		305
AF290170	281		305
AF290150	281	• • • • • • • • • • • • • • • • • • • •	305
AF176252	398		422
AF176251	398	• • • • • • • • • • • • • • • • • • • •	422
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<u>AF163904</u> AF163901	398 398		422
AF163899	398		422
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AF288524	401	•••••	425
NF288523	401 .	• • • • • • • • • • • • • • • • • • • •	425 425
<u> </u>	303		327
F123512	303		327
F206548	303		327
F197867	401		425
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AF201615			178
AF077920	154	•••••	422
<u> AF190632</u>	398		
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<u>U69845</u>	374		398
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<u>U69795</u> U69794	374	***************************************	398
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AF076048	401		425
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U37104	303		327
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U653Q1	398		744

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AF015756	303 -		327
AF015754 U76052	303 401		425
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<u>U83156</u> U83155	303		327
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AJ277672	299		323
AJ277671	299		323 14592
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AJ388459	305		329 422
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AJ245678	400		424
AJ245677	400		424
<u>AJ245676</u> AJ245675	400 400		424
AJ245674	400		424
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V48241	401 401		425
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D84203	398		122
D34636	398		
D34635	398		122 267
D12198	243		
D32126	243	******	267
D32192	398		122
AB021099	398		122
AB021096	398		422
AB021091	398		422
AB021090	398		422
A8008539	15302		15326
AB006800	398		422
L12763	260 ·		284
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L28941	398		422
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AJ010056	269		293 293
AJ010054	269		293 293
AJ010053	269 ·		293 293
AJ010052	269		293
AJ010051	269		293
AJ010050	269		293
AJ010049	269		293
AJ010048	269		293
AJ010047	269		431
X95777	407		293
AJ009879	269		293
<u>AJ010055</u>	269		327
<u>U08946</u>	303		327
U08945	303. 303		327
U08944	303		327
<u>U08941</u> U08940	303		327
X95775	303		327
X95774	303		327
X95764	303		327
AF040383	287		310 422
AF232023	400		422
AF232022	400		422
AF232021	400		344
AF157466	322 324		346
AF157465	324	*****************	346
<u>AF157464</u> AF157463	324		346
AF157460	321		343
AF231664	400		422
AF231663	400		422
AF231662	400		422 422
AF231660	400		422
AF231659	400	******************	422
AF231658	400		422
AF231657	400 400		422
AF231656	400		422
AF231644 AF231639		1	422
AF232019	400		422
AF732017	400		422
AF232014	400		422
AF318564	345		367 367
AF318561	345		166
AF118562	344		366
AF118561	344		366
AF318560	344 344		366
AFILUSSE AFILUSSU	344		3-6-6
AF318557	361		403
AE318535	361		403 403
AF318555	381		403
AFILESSS	361		

AEJL8551	381	40	13
AF318552	381	40	13
AF318551	381	40	13
AF318550	381		13
AF318549	381		13
AF318548	381)
AF238041	400		2
AF326272	400		2
AF326271	400	42	2
AF326270	400	42	2
AF126266	40C		2
AJ004340	302	n	6
AJ004264	302	n	6

Database: nt

Posted date: Mar 2, 2001 12:20 AM

Number of letters in database: 2,863,827,885 Number of sequences in database: 807,597

Lambda 0.711 1.31 1.37

Gapped

Lambda K

0.711 1.37

Matrix: blastn matrix:1 -3

Gap Penalties: Existence: 5, Extension: 2

Number of Hits to DB: 39355

Number of Sequences: 807597 Number of extensions: 39355

Number of successful extensions: 15066

Number of sequences better than 10.0: 5706

length of query: 25 length of database: 2,863,827,885

effective HSP length: 17

effective length of query: 8 effective length of database: 2,850,098,736

effective search space: 22800789888

effective search space used: 22800789888

T: 0 A: 30

X1: 6 (11.9 bits)

X2: 15 (29.7 bits) S1: 12 (24.3 bits)

S2: 16 (32.2 bits)

Table 11. BLAST analysis of primers 'mcb869' in nr database of NCBI. It demonstrates that the 3' end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer.



BLASTN 2.1.2 [Nov-13-2000]

Reference:

Altachul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:1389-1402.

RID: 984593033-24247-14777

Query-

(26 letters)

Database: nt

807,597 sequences; 2,863,827,885 total letters

If you have any problems or questions with the results of this search please refer to the ${\tt BLAST\ FAQs}$

Taxonomy reports

Distribution of 500 Blast Hits on the Ouerv Sequence

Mouse-over to show defline and scores. Click to show alignments
×
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8

Score (bits) Value Sequences producing significant alignments:

<u>52</u> 6e-06 qb[AF189111.1|AF189111 Cryptotermes austrinus cytochrome b ... 52 6e-06 qb|U868]4.1|U868]4 Phylloris wolffsohni MSB 67270 cytochrom... <u>52</u> 6e-06 gb[AF123633.1[AF123633 Perissocephalus tricolor cytochrome ... 52 6e-06 52 6e-06 52 6e-05 gb|AF127194.1|AF127194 Grallaria guatimalensis cytochrome b... 52 6e-05 52 6e-06 gb AF217828 1 AF217828 Aspidelaps scutatus cytochrome b gen...
gb AF160578 1 AF160578 Hypogeomys antimena Hant555 cytochrome
gb AF009931 2 AF009931 Archocentrus centrarchus cytochrome ... 52 6e-06 52 6e-06 52 6e-06 qb|AF091629.1|AF091629 Antilocapra americana cytochrome b (... gb[AF034967.1] Sigmoceros lichtensteinii cytochrome b gene, ... 52 6e-06 gb|AF038290.1|AF038290 Antechinus sp. cytochrome b gene, mi... gb|U07577.1|AMU07577 Antechinus melanurus mitochondrion cyt...
gb|U81343.1|CFU81343 Chelus fimbriata cytochrome b gene, mi... <u>52</u> 6e-06 <u>52</u> 6e-06 emb|AJ222631.1|ABCYTOB Alcelaphus buselaphus mitochondrial ... 52 5e-06 52 6e-06 52 6e-06 52 6e-06 gb|M99464.1|PNZMTCYTB Planigale sp. cytochrome b gene, comp... emb|AJ225116.1|DNJ225116 Dryomys nitedula mitochondrial gen... gb|UZ5738.1|PRUZ5738 Paradisaea raggiana cytochrome b gene,... qb|U25736.1|PRU25736 Paradisaea rubra cytochrome b gene, mi... 52 6e-06 <u>52</u> 6e-06 gb|U15202.1|SMU15202 Seleucidis melanoleuca mitochondrion c... 52 6e-06 52 6e-06 52 6e-06 52 6e-06 gb|U15204.1|PR15204 Paradisaea raggiana mitochondrion cytoc... emb|X56290.1|MIDDCYTB D.dama mitochondrion cyth gene for cy... emb|XS6286.1|MIAACYTBA A.americana mitochondrion cyth gene ... dbi|D88639.1|D88639 Anoa depressicomis mitochondrial DNA f... dbi|D82890.1|D82890 Bubalus depressicornis mitochondrial DN... 52 6e-06 gb | AF119261 1 | AF119261 | Peromyscus maniculatus cytochrome b ...
gb | AF123615 1 | AF123615 | Rupicola rupicola cytochrome b gene. ...
gb | AF160603 1 | AF150601 | Apodemus sylvaticus Asyl588 cytochro... 46 3e-04 46 3e-04 45 3e-04 46 3e-04 46 3e-04 46 3e-04 46 3e-04 46 3e-04 46 3e-04 gb|U62697.1|CCOLCYTB2 Charadrius collaris cytochrome b (cyt... gb|U62585.1|CBICCYTB2 Charadrius biciactus cytochrome b (cy... gb[AF022071.1] Madoqua guentheri cytochrome b (cytb) gene, ... gb[AF022070.1] Madoqua kirkii cytochrome b (cytb) gene, mit... gb|U81317.1|PSU83317 Polihierax semitorquatus cytochrome b ...
gb|U37293.1|CCU37293 Cepphus columba cytochrome b gene, mit... 45 3e-04 gb|U37292.1|CCU37292 Cepphus carbo cytochrome b gene, mitoc...
gb|U37291.1|BMU37291 Brachyramphus marmoratus perdix cytoch... 45 3e-04 45 3e-04 45 3e-04 qb|AF082055.1|AF082055 Rupicola rupicola cytochrome b gene.... gb U72770.1 JMU72770 Jabiru mycteria cytochrome b gene, mit... 45 3e-04 gb|U07578.1|OCU07578 Dasycercus cristicauda mitochondrion c... 45 3e-04 45 3e-04 45 3e-04 SD[AF031908.1[GOCCCVTB] Geopsittacus occidentalis cytochrom... emb|AJ004231.1|SBAJ4231 Sula bassana micochondrial cych gen... emb|AJ004210.1|SBAJ4210 Sula bascana mitochondrial cych gen... emb|A7004229.1|SBAJ4229 Sula bassana mitochondrial cyth gen...
emb|AJ004232.1|SBAJ4212 Sula bassana mitochondrial cyth gen...
emb|U88865.1| Pomacentrus sp. cytochrome b (cyth) gene, mito... 45 Je-04 45 3e-04 45 3e-04 45 3e-04 45 3e-04
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44 0.001 gb|U90001.1|MBU90001 Morus bassanus cytochrome b gene, mito...
gb|U63057.1|SMU63057 Brachyramphus marmoratus perdix cytoch... dbilAB036404.1|AB036404 Rana porosa brevipoda micochondrial... dbi|AB036402_l|AB036402 dbi|AB036400_l|AB036400 dbi|AB036398_l|AB036398 Rana porosa brevipoda mitochondrial... abjul9611.1|JMU19611 Jabiru mycteria cytochrome b gene, mit...
emb[X92539.1|HACYTB H.ampullatus cytochrome b gene (complet... qb[G080]4 1[GAENTC(TBA Galeocerdo cuvier mitochondrial cyto... SPICORO33.11CPLMTCTTBB Carcharhinus porosus mitochondrial C... qb[AY015012.1] Crypturellus tataupa mitochondrion, partial ... qb[AF074591_1]AF074591 Petrochelidon pytthonota cytochrome ... gb|Ar005212.1| Podepita whitii isolate 2 cytochrome b (cytb... qb[AY005211.1] Podepisa whitil isolate i cytochrome b (cytb... 44 0.07L 44 0.07L abiAP112149.11AP112149 Ovie canadensis cytochrome b gene. p ... qbiarizzis tiarizzis
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gb AF081989.1 AF081989	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081988.1 AF081988	Vireo cassinii cassinii specimen-vou	44	0.001
	Vireo cassinii cassinii specimen-vou	44	100.0
gb AF081987.1 AF081987	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081986.1 AF081986	Aileo Casainii casainii sheetmen tonti	44	0.001
gb AF081985.1 AF081985	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081984.1 AF081984	Vireo cassinii cassinii specimen-vou		0.001
gb[AF08198].1[AF08198]	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081982.1 AF081982	Vireo cassinii cassinii specimen-vou	44	
gb AF081981.1 AF081981	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081980.1 AF081980	Vireo cassinii cassinii specimen-vou	44	0.001
95 AFORISTO 1 1 AFOR1979	Vireo cassinii cassinii specimen-vou	44	0.001
95 AF081979.1 AF081979	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081978.1 AF081978	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081977.1 AF081977	Vireo Cassinii cassinii specimen vou	44	0.001
gb AF081976.1 AF081976	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081975.1 AF081975	Vireo cassinii cassinii specimen-vou	_	0,001
gb AF081974.1 AF081974	Vireo cassinii cassinii specimen-vou	44	0.001
qb AF081973.1 AF081973	Vireo cassinii cassinii specimen-vou	44	0.001
gb AF081972.1 AF081972	Vireo cassinii cassinii specimen-vou	44	
gb AF081971.1 AF081971	Vireo cassinii cassinii specimen-vou	44	0.001
GD AF081970.1 AF081970	Vireo solitarius alticola country US	44	0.001
gb AF081969 1 AF081969	Vireo solitarius alticola country US	44	0.001
gb AF081968 1 AF081968	Vireo solitarius alticola country US	44	0.001
GD AF081968.1 AF081968	Vireo solitarius alticola country US	44	0.001
gb AF081967.1 AF081967	Vireo solitarius solitarius specimen	44	0.001
gb AF081966,1 AF081966	Vireo solitarius solitarius specimen	44	0.001
gb AF081965.1 AF081965	Vireo solitarius solitarius specimen	44	0.001
gb AF081964.1 AF081964	Vireo golitarius sotitatius speciment	44	0.001
gb AF081962.1 AF081962	Vireo flavifrons specimen-voucher LS	44	0.001
gb AF081961.1 AF081961	Vireo flavifrons specimen-voucher LS	44	0.001
gb AF081950 . 1 AF081960	Vireo leucophrys leucophrys specimen	44	0.001
gb AF081959 . 1 AF081959	Vireolanius leucotis leucotis cytoch		0.001
gb AF112405.2 AF112405	Barbus anoplus cytochrome b (cytb) g	44	0.001
gb[AF144317.1[AF144317	Amphiprion ocellaris isolate 3 haplo	44	0.001
gb/AF144316.1/AF144316	Amphiprion ocellaris haplotype 3DH11	44	0.001
gb[AF144315.1 AF144315	Amphiprion ocellaris haplotype 3DH15	44	0.001
gb AF144314.1 AF144314	Amphiprion ocellaris isolate 2 haplo	44	0.001
gb AF144313.1 AF144313	Amphiprion ocellaris isolate 1 haplo	44	0.001
gb AF144312.1 AF144312	amphingion ocellaris haplotype 3DH12	44	0.001
gb AF144311.1 AF144311	Amphiprion ocellaris haplotype 3DH1	44	0.001
gb AF144310.1 AF144310	Amphiprion ocellaris isolate 2 haplo	44	0.001
-blac144309 114F144309	Amphiprion ocellaris isolate I haplo	44	0.001
FEEINC 001567.1 Bos ta	urus mitochondrion, complete genome	44	0.001
Gb AF212124 . 1 AF212124	anolis schwartzi cytochrome b gene	44	0.001
GD AF182706.1 AF182706	phanitreron amethystina cytochrome b:	44	0.001
gb AF010406.1 AF010406	Ovia aries complete mitochondrial ge	44	0.001
gb AF096452.1 AF096452	Platysteira cyanea cytochrome b gene	44	
-blas281619 11AF283619	glaphe obsoleta LSUMZ39162 cytochrom	44	0.001
IN =791618 1 AF781618	Flanke obsoleta LSUMZ H15896 cytochr	44	0.001
-blassesses 1 AF281608	Flanke obsoleta LSUMZ H14782 cytochr	44	0.001
105783602 11AF283602 ·	Flanke obsoleta LSUMZ HJ388 cytochro	44	0.001
LACTIONES TIAETIONES	etannia marrinica cytochrome b gene	44	0.001
	Actophilornis africanus cytochrome b	44	0.001
-5125271410 112F271410	Calago moholi cytochrome b (cyt b) g	44	0.001
ChiaF790139 1 AF790139	Peucedramus taeniatus cytochrome b (44	0.001
FEEINC 002504.11 Lama p	acos micochondrion, complete genome	44	0.001
ACT 61901 1 AF161901	Microrus ochroquster Cytochrome b 35	44	0.001
qb[AF11926]. 1[AF11926]	Myopus schisticolor cytochrome b gen	44	0.001
gb AF119259.1 AF119259	Synapcomys borealis cycochrome b gen	44	0.001
gb1AF288454 . L AF288454	Nyctereutes procyonoides koreensis c		0.001
gb AF153895,1 AF153895	Microcus gragalia cycochrome 8 (cyc8	-11	0.001
	Machaeropterus regulus etriolatus Cy	4.1	0.001
qb[AF12]547.1[AF12]647	Machaeropterus pyrocephalus cytochro	44	0.001
	Xenopipo atronitena cytochrome b gen Pipra fasclicauda cytochrome b gene,	44	0.001
	Pipra faecilcauda cytochrome b gene Pyroderus scutatus cytochrome b gene	프	0.001
95 AF123534 . 1 AF123534	Pyroderus scutatus cytochrome b gene Cephalopterus ornatus cytochrome b g	41	0.001
	Turdampelle cryptolophue cytochrome	-11	100.0
	Porphyrolasma porphyrolasma cycochro	4 4	0.001
3 7-1	Ampelioides technicali cytochrome b gr	4 4	0.001
SPIASIZIKIS LIASIZIKIS	piacegle chlorolopidote cytochrome b	4.5	0.001
CHIAFIZISIA LIAFIZISIA	Rupicola persylana cytochrome b gene	_•.•	0.071
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gb[AF127201.1]AF127201	Myrmothera campanisona cytochrome b	44	0.001
qb AF127192.1 AF127192	Grallaria ruficapilla cytochrome b g	44	0.001
GD[AF12/192.1[AF12/192	Grallaria varia cytochrome b gene, p	44	0.001
qb AF127189.1 AF127189	Grailaria varia cycochrome o gene, p	44	0.001
gb AF197849.1 AF197849	Sericornis frontalis cytochrome b ge	44	0.001
gb AF197847.1 AF197847	Pardalotus striatus cytochrome b gen		0.001
elyc onnegg 1 Winno	noramis amphibius mitochondrion, comp	44	0.001
flac 002079 11 Caras	ging auratus mitochondrion. Complete	44	
FINC 001794 11 Macto	ous robustus mitochondrion. complete	44	0.001
Tef NC 001610.1 Didel	obje virginiana mitochondrion, comple	44	0.001
- La Transcata 1 A 5301613	Stomatorhinus sp. CU79703 cyotchrome	44	0.001
gb AF201612.1 AF201612	Amphiprion clarkii cytochrome b gene	44	100.0
qb AF097931.1 AF097931	Amphiprion ocellaris cytochrome b ge	44	0.001
gb AF097927.1 AF097927	Amphiprion desirates excelete denome	44	0.001
qb J01194.1 BOVMT Bos	taurus mitochondrion. complete genome	44	100.0
gb AF168760.1 AF168760	Apalone spinifera isolate TXSC cytoc	44	0.001
Gb AF168759.1 AF168759	Apalone spinifera isolate TXki cytoc		0.001
gb AF168758.1 AF168758	Apalone spinifera isolate TXcc cytoc	44	0.001
gb AF168756.1 AF168756	Apalone spinifera isolate NMrg cytoc	44	0.001
95 AF182381.1 AF182381	perrochelidon rufocollaris isolate E	44	
Lagrange 1 AFT 82380	Perfochelidon rufocollaris isolate E	44	0.001
gb U89187.1 MMU89187 M	omorus mericanus cytochrome b (cytb)	44	0.001
gb AF193833.1 AF193831	Rotaumia lenticinosus cytochrome b g	44	0.001
GD AF193833 , 1 RF193833	Ardea alba cytochrome b gene, partia	44	0.001
gb AF193822.1 AF193822	Ardea herodias cytochrome b gene, pa	44	100.0
gb AF193821.1 AF193821	Paranaja multifasciata cytochrome b	44	0.001
gb AF217837.1 AF217837	Naja kaouthia cytochrome b gene, com	.44	0.001
gb AF217835.1 AF217835	Laticauda colubrina cytochrome b gen	44	0.001
gb AF217834.1 AF217834	Calliophis japonicus cytochrome b ge	44	0.001
gb AF217831.1 AF217831	Calliophia Japonicus cycomicus o 30	44	0.001
gb AF217823.1 AF217823	Micruroides euryxanthus cytochrome b	44	0.001
ab AF217819.1 AF217819	Drysdalia coronata cytochrome b gene	44	0.001
gb AF217815.1 AF217815	Austrelaps superbus cytochrome b gen	44	0.001
gb AF118156.1 AF118156	Terenura humeralis specimen-voucher	44	0.001
gb AF209938.1 AF209938	Euura atra isolate 62 cytochrome b g	44	0.001
gb AF209933.1 AF209933	Euura atra isolate C cytochrome b ge	44	0.001
gb AF059104.1 AF059104	Marmaronetta angustirostris cytochro	44	0.001
gb AF059102.1 AF059102	Lophonetta specularoides cytochrome	44	0.001
GD AF059054 . 1 AF059054	Amazonetta brasiliensis cytochrome b	44	0.001
gb AF192646.1 AF192646	Hippocampus barbouri haplocype PH.22	44	0.001
gb AF192645.1 AF192645	Rippocampus barbouri haplotype PH.13	44	0.001
gb AF160614.1 AF160614	Cricetomys gambianus Cgam518 cytochr	44	0.001
gb AF160613.1 AF160611	Cricetomys emini Cemi531 cytochrome	44	0.001
gb AF160612.1 AF160612	Cricetomys emini Cemi530 cytochrome	44	0.001
qb[AF160611.1[AF160611	Cricetomys emini Cemi637 cytochrome	44	0.001
gb AF160610.1 AF160610	Cricetomys emini Cemi636 cytochrome	44	0.001
gb AF160604,1 AF160604	Calomyscus bailwardi Cbal576 cytochr	44	0.001
gb AF160560.1 AF160560	Eliurus majori Emaj642 cycochrome b	44	0.001
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gb AF160558.1 AF160558	Eliurus majori Emaj639 cytochrome b	44	0.001
gb AF160557.1 AF160557	Eliurus majori Emaj638 cytochrome b	44	0.001
gb AF160555.1 AF160555	Eliurus majori Emaj614 cytochrome b	44	0.001
95 AF160554 . 1 AF160554	Eliurus majori Emaj617 cytochrome b	44	0.001
gb[AF160553.1[AF16055]	Eliurus majori Emaj571 cytochrome b Eliurus majori Emaj556 cytochrome b	44	0.001
951AF160552.11AF160552	Eliurus majori Emajori cytochrome b	44	0.001
gb AF160551.1 AF160551	Eliurus majori Emajidi cycochrome b	44	0.001
95 AF160550 . LIAF160550	Eliurus majori Emaj444 cytochrome b	44	0.001
95 AF160549 . L AF160549	Damaliscus pygargus cytochrome b (cy	44	0.001
95 AF016287. 1 AF016287	Oryx leucoryx cytochrome b (cytb) ge	44	0.001
95 AF016286. LIAF016286	Antilope cervicapra cycochrome b (cy	44	0.001
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95 AF108682 . LIAF108692	shinidomys nicela cycochrome 8 (cyc8	44	0.001
qb[AF10867]. [[AF10967]	Thomasomus dichie cycochioms B (cycb	44	a.a11
901AF108552.11AF109562	Scapteromye tumidue cytochrome B lcy	44	0.001
951AF942729.11AF942729	Magamuntiacus vuquangensis cycochrom	33	0.001
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gb AF084074.1 AF084074 Lagenorhynchus albirostris cytochrom	44	0.001
gb AF090750.1 AF090750 Gobio gobio balcanicus cytochrome b	44	0.001
gb AF157919.1 AF157919 Spermophilus columbianus columbianus	44	0.001
gb AF157917.1 AF157917 Spermophilus washingtoni isolate 589	44	0.001
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qb AF157819.1 AF157839 Spermophilus elegans elegans isolate	44	0.001
gb AF030497.1 AF030497 Crocidura brunnea cytochrome b (cyt	44	0.001
qb U03541.2 LAU03541 Lenoxus apicalis cytochrome b gene, pa	44	0.001
gb A2009951.2 AF009951 Heros appendiculatus cytochrome b (c	44	0.001
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gb AF094621.1 AF094621 Eminia lepida cytochrome b gene, par	44	0.001
gb AF094618.1 AF094618 Hypergerus atriceps cytochrome b gen	44	0.001
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gb AF034724.1 AF034724 Pantholops hodgscni cytochrome b (cy	44	0.001
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Alignments

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AF189111	797		772
U86834	858		833
AF123633	56		31
AF123617	104		79
AF127202	107		82
AF127194	107		82
AF217828	845		820
AF160578	869		844
AF009931	869		844
AF091629	869		844
AF034967	869		844
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AF119261	869	*******	847
AF123615	101	*****	79
AF160603	866		844
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U62685	179	• • • • • • • • • • • • • • • • • • • •	157
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AJ004229 AJ004232	773 773		751
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U90001	536		514
U63057	773		75 L
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AB036403	173	•••••	151 151
AB036400	173 173	*****************	151
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AF144312	161	gg	136 136
AF144311	161	p	136
AF144310 AF144309	161 161		136
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<u>AP146616</u> AF271410	95 869	gg	844
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AP119261	869 869	**************************************	844
AP119759 AP788454	91		66
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P121617	68 104	g	43 77
P121616	66		43
FIZICIA	64		59

			70
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AF127189	107	ggg.	82_
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AF168756	507 692		667
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			12001
NC DOLP41 APIDE698			***

,**1**

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AF157912	869	******
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AF157882 AF157859	869	a 844
AF157858	869	
AF157839	869	g844
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003541	869	g 844
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AF094618	737	712 844
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AF022059	869 869	
AF022058 AF022057	869	g
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AF096624 AF081052	869 869	a844
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argazagi	672	647
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U17860	869		775
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AF034728	869	gg	844
AF034727	869	a	844
AF034724	869	gg	844
AF034722	869	gg.	844
U72038	869	gg	844
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AF082047	869		844
AF038286 AF038284	869		844
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AF059091	776		751 751
AF059080	776		751
AF059078	776		36
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AF006227	774	gg	749
AF047447	41	g	16
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AF028180	65	gg	40
AF028178	69	gg	52
AF028170	77 869		844
<u>M99454</u> M99451	869		844
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<u>007590</u>	869	g	748
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AF020255 Y19184	15022	gg	14997
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<u> </u>	843	e	838
<u>U88857</u>	863 867	gg	842
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U81157	79L		766
U81136	791		766 427
<u>175354</u>	452 672	· · · · · · · · · · · · · · · · · · ·	847
U77112 229571	15045		12030
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A1722580	669		844
#1222683	469	· · · · · · · · · · · · · · · · · · ·	•••
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			847
V15201	872		847
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			715
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	774		749
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X74260	872	gg	847
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X74256	869	g	844
X82304	869		844
X82302	869		844
X56284	872		847
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X74259	872	G	844
Y08814	869		844
Y08813	869	gg	844
<u>x56287</u>	869		847
X74251	872	·····g······	748
X60941	773		847
X74255	872	g	844
X56289	869	gg	15357
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X92530	869 774		749
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D88612	869		844
088630 .	869		844
068628	869		844
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092922	169		444
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012121	467	*****	• • •

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D84203	869	· · · · · · · · · · · · · · · · · · ·	844
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D82894	869	aa	844
D82891	869	· · · · · · · · · · · · · · ·	844
D34638	869		844
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AB037602	869	aa	844
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AB004070	869		844
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U10367	773	gg	748
U10364	773	gg.	748
Z96068	869	g	844
076507	773	·····	749
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AF157464	793		774 774
AF157463	793		74
AY016019	93	•••••	850
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AF027324	869		850
AF027121	869		850 850
AF027322	869 869		850
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AF027316	869		850
AF027315	869	*************	850
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AF027109	869		850
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AF266188	371		352
AF324034	408		389
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AF272614 AF272611	867		850
AP182711	712		693
AP107697	774		755
AF155122	669		850
AF155199 AF155185	669 669		850
W172787	669		450
0E133332	969		430
4212222	467		630
82133372	467	•••••	

AF155589	669		850
AF155588	869		850
AF123531	771		752
AF264047	869		850
AF206531	771	******	752
AF192706	863		844
NC 000884	15032	***************************************	15013
AF004572	869		850
AF088912	869 176	***************************************	850 157
<u>U62697</u>	179	gn	160
<u>U62681</u>	179		154
<u>U62707</u> AJ004315	773		748

Database: nt

Posted date: Mar 2, 2001 12:20 AM

Number of letters in database: 2,863,827,885 Number of sequences in database: 807,597

K Lambda 1.37 0.711 1.31

Gapped

K H Lambda

1.37 0.711 1.31

Matrix: blastn matrix:1 -3

Gap Penalties: Existence: S. Extension: 2

Number of Hits to DB: 19068 Number of Sequences: 807597 Number of extensions: 19068

Number of successful extensions: 7580

Number of sequences better than 10.0: 2441

length of query: 26 length of database: 2,863,827,885

effective RSP length: 17

effective length of query: 9

effective length of database: 2,850,098,736

effective search space: 25650888624 effective search space used: 25650888624

T: 0 A: 30

X1: 6 (11.9 bits)

X2: 15 (29.7 bits) S1: 12 (24.3 bits)

\$2: 16 (32.2 bits)

Table 12. The other animals belonging to distantly related species analyzed by our primers to demonstrate its universal nature

SN.	Name of the animal
1.	Indian black buck no. l
2.	Indian black buck no 2
3	sheep
4	pig
5	dog
6	chimpanzee (chimss)
7	human (humsk)
8	Hamster
9	crocodile no l
10	crocodile no2
11	turtle no l
12	turtle no2
13	mouse
14	varanus
15	Naga-naga snake
16	Indian elephant
17	hen
18	dugong
19	lizard
20	weaver bird no l
21	weaver bird no2
22	buffalo no l
23	buffalo no 2

CLAIMS

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Universal primers named as 'mcb 398' and 'mcb 869' capable of amplifying a fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and revealing the identity of the biological material of any animal of unknown origin at species and sub-species level, said primers, having the sequences:

primers name Sequence (5'-3')
mcb 398 "TACCATGAGGACAAATATCATTCTG"
mcb 869 "CCTCCTAGTTTGTTAGGGATTGATCG

- 2. Primers as claimed in claim 1 wherein the fragment of mitochondrial cytochrome b gene is capable of significantly discriminating amongst various evolutionary lineages of different animal species.
 - Primers as claimed in claim 1 wherein the fragment of mitochondrial cytochrome b
 gene is flanked by the highly conserved sequences amongst a vast range of animal
 species.
- 4. Primers as claimed in claim 1 wherein the fragment on mitochondrial cytochrome b gene which is polymorphic inter-specifically, but monomorphic at intra species sources.
 - 5. Primers as claimed in claim 1 wherein in Antilope cervicapra species, the sequences of the fragment mentioned under claim 1 are as follows:
- 20 Mitochondrial cytochrome b gene sequence (398-869 bp) of Antilope cervicapra:

 "taccatgaggacaaatatetttttgaggagcaacagtcatcaccaateteetttcagcaateccatacateggtacaaacetag
 tagaatgaatetgaggagggttetcagtagataaagcaaceettaceegatttttegeetteeaetttateeteecatttateattge
 agecettaccatagtacacetactgttteteeaegaaacaggateeaacaaceecacaggaateteatcaagacgcagacaaa
 atteeatteeaeeetactacaactateaaagatateetaggagetetactattaattttaaeeeteatgettetagteetattetaeee
 25 ggacetgettggagaceeagacaactatacaceagcaaaceeaettaatacaceecacatateaageeegaatgatactte
 ctatttgeatacgcaateeteegatcaatteetaacaaactaggagg"
 - 6. A method for the identification of the animal from a biological sample, said method comprising the steps of:
 - a) isolating and amplifying the DNA from the biological sample to be tested using the primers as claimed in claim 1,
 - b) sequencing the amplified products,
 - c) blasting the sequence resolved in step (b) against mito database of National Centre for Biotechnology Information (NCBI) using BLAST program and determining the most likely family of the animal source of the biological sample.

- d) blasting the sequence resolved in step (b) against non-redundant (nr) database of National Centre for Biotechnology Information (NCBI) using BLAST program and determining the most likely genus, species or more precisely the sub-species of the animal source of the biological sample,
- e) identifying the most significant alignment of the sequence resolved with cytochrome b gene sequence of the animal identified in steps (c) and (d) respectively and selection of these animals as 'reference animals' for further studies,

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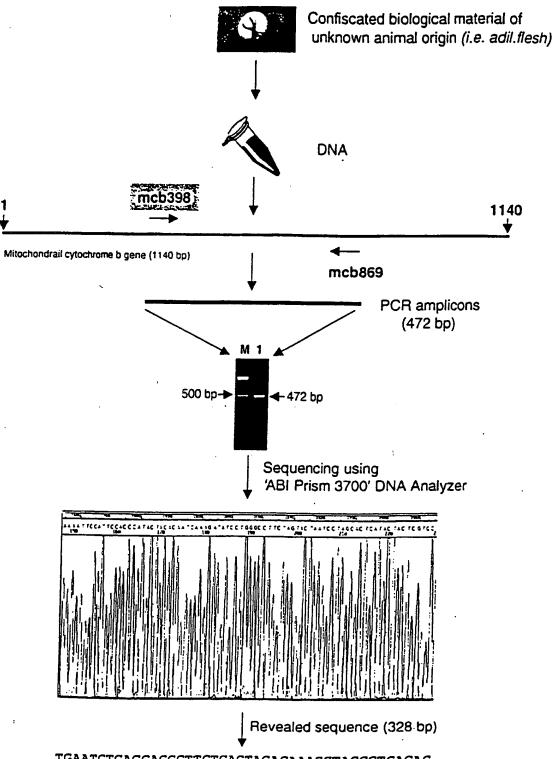
- f) isolating and amplifying and sequencing the DNA sequences from the reference animal on both strands in triplicate using the primers as claimed in claim 1,
- g) aligning the sequences obtained using CLUSTRAL program and identifying the variable sites amongst the animals analyzed,
- h) comparing the nucleotide sequences pair-wise to determine the variation among the animals resolved and identifying the nucleotide sequence to which the DNA sequence of the biological sample bears maximum similarity as the source animal of the biological sample.
- 7. A method as claimed in claim 6 wherein the universal PCR protocol works universally with the DNA template of any unknown animal origin and the universal primers mentioned under column 4.
- 8. A method as claimed in claim 6 wherein the Amplification reactions should be carried out in 20 μl reaction volume containing approximately 20 ηg of template DNA, 100μm each of dNTPs, 1.25 pmole of each primer, 1.5mM MgCl₂, 0.5 unit of Ampli*Taq* Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed should be: an initial denaturation at 95°C for 10 min, followed by 35 cycles each of denaturation at 95°C for 45 s, annealing at 51°C for 1 min, and extension at 72°C for 2 min. The extension step at 35th cycles should be held for 10 min.
- A method as claimed in claim 6 wherein the method enables identification of species
 of analyzed material (i.e. the DNA isolated from confiscated animal remain of unknown origin) using the public databases such as GenBank, NCBI etc.
 - 10. A method as claimed in claim 6 wherein the method is used for animal identification to establish the crime with the criminal beyond a reasonably doubt.
 - 11. A method as claimed in claim 6 wherein the method is used to establish the identity of

- biological materials such as skin, horns etc confiscated from animal poachers, if it is that of an endangered species.
- 12. A method as claimed in claim 6 wherein the method is used for establishment of the identity of confiscated animal parts and products of endangered animal species for the purpose of production of molecular evidence of animal hunting and related crime in the court of law, so that the human violation of the wildlife resources could be controlled.

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- 13. A method as claimed in claim 6 wherein the method is used to have an idea of the geographical location of the commitment of wildlife crime based on the cytochrome b gene haplotype of poached animal identified by the universal primer invented.
- 14. A method as claimed in claim 6 wherein the method is used for animal identification to detect the adulteration of animal meat in food products for the purpose of food fortification, by the food fortification agencies.
 - 15. A method as claimed in claim 6 wherein the method is used to provide a universal technique for detection of the origin of blood or blood stains etc collected from the scene of crime related to offences such as murder, rape etc, in order to establish the origin of blood found at scene of crime when it sounds as if criminals have wontedly spread the blood of an animal at the scene of crime, to confuse the crime investigation agencies and forensic scientists with human blood.
- 16. A method as claimed in claim 6 wherein the method is used so that it can be converted
 to a (a) <u>COMMERCIAL 'MOLECUALR KIT'</u> and (b) '<u>DNA CHIPS'</u> based applications for wildlife identification in forensics.



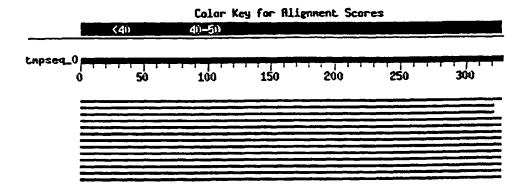
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AACAACCCCTCAGGAATAGTATCCGACTCAGACAAAATTCCAT
TCCACCCATACTACACAATCAAAGATATCCTGGGCCTTCTAGT
ACTAATCCTAGCACTCATACTACTCCTCCTATTCTCACCAGAC
CTGTTAGGAGACCCCGATAACTACATCCCTGCCAACCCTCTAA
ATACCCCTCCCCATATCAAGCCTGAAT

Figure 1 a

Sequence of cytochrome b gene (328 bp) revealed from biological material of unknown origin i.e. 'adil.flesh' using primers 'mcb398' and 'mcb869'

Homology search in 'nr' database using 'BLAST'

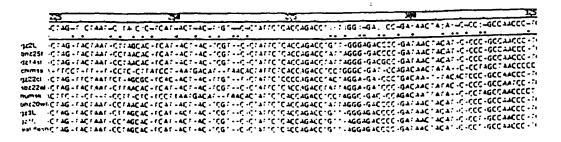
http://www.ncbi.nlm.nih.gov/BLAST/



	Score	=
Sequences producing significant alignments:	(bits)	Value
gb AY005809.1 Panthera pardus cytochrome b gene, partial c	. 603	e-170
gb AF053054.1 AF053054 Panthera tigris sumatrae isolate Su1		e-147
gb AF053053.1 AF053053 Panthera tigris tigris isolate B7 mi	527	e-147
gb AF053050.1 AF053050 Panthera tigris corbetti isolate C2	476	e-132
gblAF053049.1\AF053049 Panthera tigris corbetti isolate C1	476	e-132

Selection of reference animals based on above information and further analysis using primers 'mcb398' and 'mcb869'

Multiple sequence alignments using 'Autoassembler'



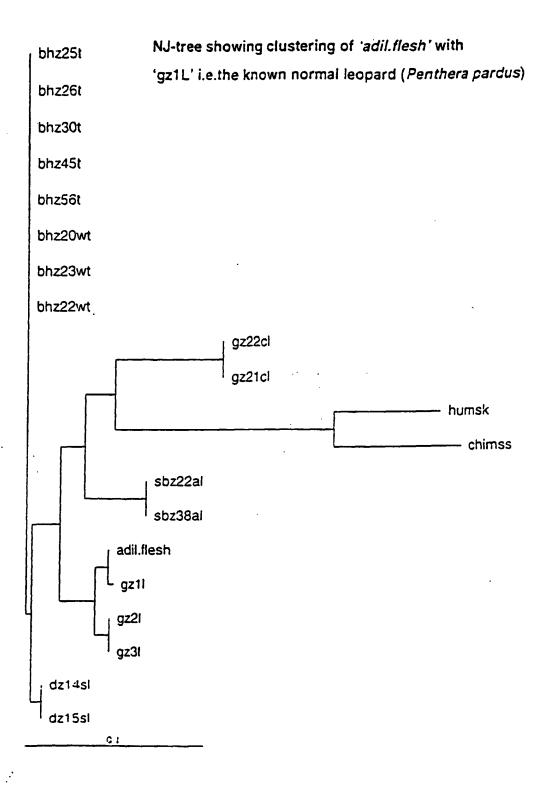


Figure 1c

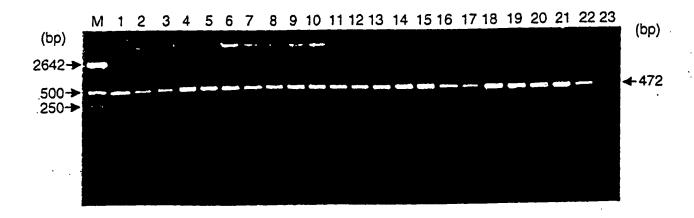


Figure 2

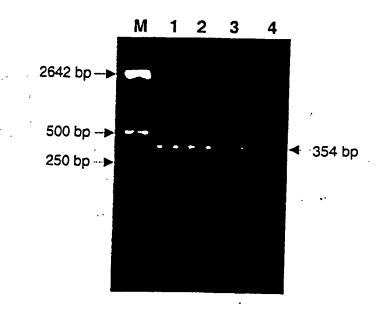


Figure 3

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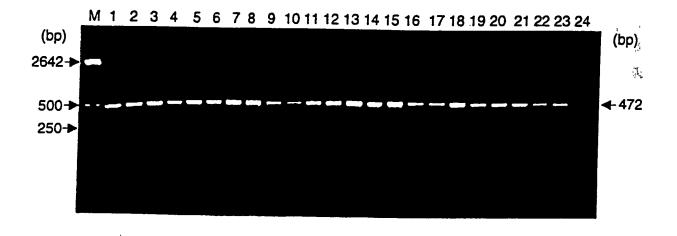


Figure 4

INTERNATIONAL SEARCH REPORT

PCI/IN 01/00055

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, EMBL, WPI Data, PAJ, MEDLINE, EMBASE

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Y	KOCHER T D ET AL: "DYNAMICS OF MITOCHONDRIAL DNA EVOLUTION IN ANIMALS AMPLIFICATION AND SEQUENCING WITH CONSERVED PRIMERS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 86, no. 16, 1989, pages 6196-6200, XP002189444 1989 ISSN: 0027-8424 the whole document	1-16
Y	WO 92 05277 A (DAVIDSON WILLIAM SCOTT; BARTLETT SYLVIA ERNESTINE (CA)) 2 April 1992 (1992-04-02) the whole document/	1-16

X Further documents are listed in the continuation of box C.	Patent family members are listed in annex.
Special categories of cited documents: 'A' document defining the general state of the art which is not considered to be of particular relevance 'E' earlier document but published on or after the international filing date 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) 'O' document referring to an oral disclosure, use, exhibition or other means 'P' document published prior to the international filing date but later than the priority date claimed	 "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention. "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone. "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family
Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2002	12/03/2002
Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

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